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ANATOMIC EFFECTS OF THE VIRUSES OF PIERCE'S DISEASE AND PHONY PEACH[1]

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INTRODUCTION

PREVIOUS INVESTIGATIONS have shown that in its method of transmission Pierce's disease virus is closely associated with the xylem of the hosts (Houston *et al.*, 1947).[3] Furthermore, the external symptoms of the diseases caused by this virus—Pierce's disease of the grape and dwarf disease of alfalfa—suggest a disturbance in the water-conducting system (Hewitt *et al.*, 1942*b*; Weimer, 1931). At present no other virus is known to be so definitely associated with the xylem as that of Pierce's disease. A study of the pathological anatomy of the hosts of this virus is therefore of particular interest in the problem of tissue relations of viruses. The investigation reported in this study was undertaken to determine whether the anatomic symptoms occur in the xylem and to what extent, if at all, they are restricted to this tissue. An attempt was also made to determine the nature and location of the first signs of histopathological disturbances. This was done through developmental studies on infected grape seedlings and cuttings of alfalfa.

Certain viruses cause degenerative changes in the xylem, without necessarily being limited to this tissue. Among these, the psorosis virus is particularly well known. The anatomy of the affected wood of citrus has been described (Fawcett and Bitancourt, 1943; Webber and Fawcett, 1935). Phony peach virus also appears to cause disturbances in the xylem (Hutchins, 1933), but the information concerning its anatomic effects on this tissue is not available. The pathological anatomy of phony peach roots has been briefly considered in the present study in order to obtain additional information on the effect of viruses on the xylem.

REVIEW OF LITERATURE

Pierce's disease of the grapevine was recognized first as a graft transmissible virus disease (Hewitt, 1939); then several of its insect vectors were discov-

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[3] See "Literature Cited" for complete data on citations, referred to in the text by author and date of publication.

ered (Hewitt *et al.*, 1942*a*). The identification of alfalfa dwarf as a virus disease transmitted through root grafting was made independently of the studies on Pierce's disease of the grapevine (Weimer, 1936). Later, alfalfa dwarf and Pierce's disease were found to be caused by the same virus and transmitted to the two hosts by the same insect vectors (Hewitt and Houston, 1941; Houston *et al.*, 1942; Hewitt *et al.*, 1946).

The external symptoms of Pierce's disease of the grapevine, particularly on *Vitis vinifera,* have been considered in detail by Hewitt *et al.*, (1942*b*). Briefly, these symptoms are as follows. Delayed growth in the spring; mottling and, later, burning and scalding of leaves; wilting and drying of fruits; dwarfing of parts or of entire vines; and uneven maturation of canes. Sudden wilting is characteristic of young, vigorously growing diseased vines, and a drying of many leaves or parts of leaves follows this wilting. Young and old vines die from the disease, sometimes in the first season of infection or one or more years later.

According to Weimer (1931) and Weimer and Madson (1932), alfalfa plants affected by the dwarf disease are, as the name implies, dwarfed in appearance, because they have short stems and rather small leaves. After each cutting, the stems become shorter and more slender and the leaves smaller. The number of stems also diminishes. Often the plants are an unusually dark green and, in the late stages of the disease, the tips of the stems sometimes wilt even in wet soil. There is, however, no mottling or crinkling or any other pronounced deformation of leaves. Blossoming is often delayed or completely inhibited.

Pierce's disease virus occupies a unique place among the known plant viruses. Whereas viruses thus far have been divided into those that are more or less localized in the phloem or in the parenchyma outside the phloem and those that are not limited to any tissue and occur in both the phloem and the parenchyma (Bennett, 1940), Pierce's disease virus is closely associated with the xylem. This relation has been clearly revealed by observations on the mode of vector feeding and on the tissues involved in the transmission of the virus (Houston *et al.*, 1947).

All the insect vectors of Pierce's disease virus, whether kept on the grapevine or on alfalfa, seek out the xylem in feeding, and the virus can multiply and cause the disease only when the xylem tissue is actually reached by the vector. Experiments on alfalfa have shown that occasionally the virus moves upward quite rapidly. This observation and the information that insects puncture the walls of the tracheary elements in feeding on the xylem (Houston *et al.*, 1947) suggest that the virus can move with the water stream in the water-conducting elements of the xylem.

In agreement with the conclusion just mentioned, some of the external and internal symptoms of the disease in the grape and alfalfa indicate that xylem is the tissue primarily affected by the disease. The sudden wilting of vigorously growing young vines and the scalding and drying of leaves in the grapevine, the wilting of stem tips and the dark coloration of the foliage in alfalfa point toward insufficient delivery of water to the tops of the plants. According to the early histologic studies on Pierce's disease, the wood of affected grapevines is discolored and gum and tyloses occur in the tracheary

elements (Dowlen, 1890; Viala, 1893; Butler, 1910). In dwarf alfalfa, also, the vessels are plugged with gum (Weimer, 1931, 1936).

For comparison with the results of the present study, the references dealing with the histology of plants affected with Pierce's disease virus are here reviewed in some detail. Dowlen (1890) appears to have been the first to report on the discoloration of the wood of affected grapevines, the filling of cell cavities with black-brown deposit, and the development of tyloses—sometimes very abundant—in the vessels. He also found moisture and starch to be deficient in the canes that failed to ripen, or in the immature areas of canes that ripened unevenly. The small amount of starch present in unripened areas did not readily turn blue on application of iodine, the individual grains were small and seemingly partly eroded. Dowlen compared Pierce's disease with mal-nero of Sicily and Italy, which also causes starch deficiency in stems and the appearance of brown matter and of tyloses in the vessels.

Butler (1910) has explained that the retention of the green color in the immature areas of the cane results from nondevelopment of a cork layer that normally cuts off the chlorophyll-containing cortex in a maturing cane of a healthy plant (see Esau, 1948*a*). In the mature areas cork is formed, however. Butler also observed starch deficiency in immature canes, but was in error when he assumed that the uneven thickness of the vascular tissues in the different parts of the circumference of a cane—a feature characteristic of grape canes (see Esau, 1948*a*)—resulted from the presence of the disease. Butler mentioned tyloses in primary and secondary wood, and gumlike deposits in cells of all tissues of the cane, but the main object of his histologic study was the mottled leaf.

According to Butler (1910), the degenerative changes vary in different parts of the affected leaves. In the chlorotic areas, chloroplasts degenerate. They become less discrete and aggregate into plasmodia-like masses, which finally disappear. Such degeneration is particularly characteristic of the palisade tissue. In the spongy layers plastids may become vacuolate, but do not distend, and either fragment or degenerate into oil-like bodies. The parts of leaves showing red discoloration, on the other hand, have an overabundance of starch in the chloroplasts. When the reddened areas die, starch disappears, and the cells become filled with a morphologically variable occluding matter. Different amounts of this substance occur also in cells that are not dead. Microchemical reactions of the occluding matter suggested to Butler that the latter was composed of tannin, proteinaceous materials, and wound gum. The absence of occluding matter in the chlorotic areas where the plastids degenerated completely, and its presence in parts where starch was plentiful before the appearance of this matter, indicated to Butler that the occluding substance was derived mainly from decomposed starch. Various degrees of occlusion of vessels by the gumlike substance or by tyloses also occurred in the leaves.

Butler (1910) found no symptoms of diagnostic value in the arms, trunks, and roots of the grapevine, and concluded that Pierce's disease primarily affected the leaves, fruit, shoots, and canes.

The only symptom of alfalfa dwarf observed in the roots was the deposition of gum in the vessels (Weimer, 1931, 1936; Weimer and Madson, 1932).

According to Weimer (1931), in the earliest stage of disease development the wood becomes yellow just beneath the bark. This yellowing, which results from the appearance of gum in the vessels, spreads from the outer portion of the root toward the interior. Frequently, when the plant dies, the root is discolored throughout. During the winter and early spring, the discoloration does not extend to the cambium but is separated from it by a layer of new wood. Weimer thinks that this distribution of the symptom indicates inactivity of the disease during the winter months. The yellow discoloration occurs also in the main divisions of the crown and in the bases of the green stems, but apparently does not extend far up into the latter.

The gum accumulation in vessels, although characteristic of plants infected with Pierce's disease virus, is not a specific symptom of this malady. It may be caused in alfalfa and the grapevine (as well as in other plants) by other pathogens and certain physiological disturbances. The bacterium *Aplanobacter insidiosum* L.Mc., the causal agent of alfalfa wilt, induces symptoms in alfalfa roots, which are very similar to symptoms in plants affected with alfalfa dwarf. The invasion of the vessels by the bacteria is followed by deposition of gum in these elements (Jones and McCulloch, 1926). Later, the gumlike material appears also in vessels beyond those invaded by the bacteria (Jones, 1928*b*). The deposition of gum is accompanied by a change in the composition of the walls of groups of cells adjoining the vessels, as is evidenced by their brilliant staining with phloroglucinol and hydrochloric acid (LeClerg and Durrell, 1928).

A similar plugging of vessels in alfalfa roots may be induced by a high water content in the soil and by injections of various salt solutions (LeClerg and Durrell, 1928). Jones (1928*a*) found gum formation in the vessels of alfalfa after winter injury to the roots and after injection of dilute acids through the cut ends of the stem. Alfalfa roots affected with the wilt disease are low in starch. The depletion of the latter is obviously related to the plugging of vessels: the starch disappears first of all near these vessels (Peltier and Schroeder, 1932). Tyloses appear to be rare in alfalfa roots (Jones, 1928*a;* LeClerg and Durrell, 1928).

The grapevine also produces gum and tyloses in the xylem under varied conditions, some of which may not be abnormal (Mangin, 1895; Ráthay, 1896). Ráthay studied gum formation in grapes in particular detail, and used more than one species of *Vitis* and even other representatives of the *Vitaceae*. He found that in all species of *Vitis* and in all cultivated varieties which he had studied, the two- to several-year-old growth of the aboveground parts contained some vessels with gum. In one variety of *Vitis vinifera* he found gum in one-year-old wood also. Gum development in roots appeared to be less common, less regular, and later than in the stem parts. The sapwood, according to Ráthay, is transformed into heartwood between its twentieth to thirtieth years. Gum formation is not associated with this change. In fact, as the heartwood is formed, tyloses develop in vessels containing gum.

Ráthay also studied gum formation caused by wounding. Below transverse cuts of young shoots (one to two years old) the tissues died part way. Beneath the dry dead part, vessels became clogged with gum and, still lower, tyloses developed in the same vessels that contained gum at the higher level.

Some workers have given considerable attention to the nature and origin of gum in plants. The grapevine gum, according to Ráthay (1896), gives the reaction of pentoses, has a strong swelling capacity, and contains over 50 per cent of water. Ráthay was of the opinion that gum was formed in the xylem-parenchyma cells, but that it probably left these cells as a slowly diffusing colloid and only then assumed the nature of gum. He decided that the gum occurred under pressure in the vessels—hence, its exudation from cut branches—and this pressure did not change with the seasons.

According to Tschirch (1889), plant gum is derived from decomposition of cell walls, but is also apparently produced by living cells. Butler (1911) strongly emphasized that cell contents, including the starch, were in no way concerned with gum formation in *Prunus* and *Citrus*: the gum resulted solely from the hydrolysis of walls. (In the grape, however—Butler, 1910—as stated previously, associated the gummy deposit in the mesophyll with starch decomposition.)

Bartholomew (1928), on the other hand, considered that in endoxerosis of lemons both starch and cell wall were the sources of gum. He described a swelling of the middle lamella, in tissues other than the xylem, in the early stages of gummous degeneration, then a breakdown of the rest of the wall. When gum developed in the xylem, the middle lamellae of the walls between the parenchyma cells and vessels were affected first, then the cellulose wall, and finally the starch in the parenchyma cells. The walls, however, were not completely destroyed. Bartholomew observed accumulations of gum in the pit cavities on the parenchyma side. This gum then passed through the pits, and appeared as droplets on the inner face of the vessel wall. The droplets later coalesced and filled the vessels. The gum had variable properties during the different stages of its development. At first it was water soluble, and remained thus for at least a week or 10 days after it migrated into the vessels. Later it became less soluble and finally entirely insoluble in water. Bartholomew suggested that perhaps only at this last stage should the substance be referred to as "gum." Since the processes of conversion of the pectic substances, cellulose, and starch into water soluble or insoluble gums are highly complex, many intermediate substances are formed before the endproduct is reached. This complexity explains the variability in the staining reactions and properties of the gum at the different stages of its development.

Peltier and Schroeder (1932) suggested that the gum in alfalfa roots affected with wilt might be the residue (possibly lignin) left after the bacteria have dissolved out the soluble pectins from the middle lamella.

The virus disease of citrus called "psorosis" (Fawcett and Bitancourt, 1943; Bitancourt *et al.*, 1943) induces, among other symptoms, gum deposition in the vessels, which is associated with starch depletion in the surrounding cells. The authors, therefore, assume that the gum is derived from the starch.

A summing up of the literature on gummosis just reviewed indicates that gum deposition and tylose development in the tracheary elements of the xylem are frequent phenomena. They may occur in the xylem of various plants under apparently normal conditions, but particularly when the tissue is affected by various pathogens and physiological disturbances. Most workers agree that the gum is derived from decomposition of carbohydrates—notably

starch—and that the products of decomposition migrate from the living cells into the tracheary elements. Most often, the gum has been identified—in its final state of transformation—as a wound gum giving lignin reaction.

Phony disease of peach causes a dwarfing of branches, twigs, and fruit of the affected tree. With each succeeding year the amount and quality of the fruit are reduced. The disease, however, does not kill infected trees, nor does it cause any yellowing of the leaves. On the contrary, the foliage of the diseased trees appears more vigorous than that of the healthy (Hutchins, 1933).

The only known internal symptom of the disease is the appearance of well-distributed purplish spots in the wood of fresh roots treated with acidulated absolute methyl alcohol (Hutchins, 1933). Since Hutchins (1933) was able to transmit the virus by grafting of roots but not of tops, he concluded that the virus did not invade the branches of the tree. Moreover, Hutchins (1939) obtained evidence suggesting that the virus was localized in the woody cylinder of the root: the virus was transmitted if whole root sections were used in grafting, but not if the sections contained only the bark.

MATERIAL AND METHODS

The grapevine (*Vitis vinifera* L.) and alfalfa (*Medicago sativa* L.) material was obtained partly from plants grown and inoculated with Pierce's disease virus in a greenhouse at Davis, and partly from field-grown plants naturally infected with the virus in various localities of California. Healthy material was available in the same locations as the diseased. The inoculations in the greenhouse were made by using the insect vectors of the virus. The healthy and phony-diseased peach samples were imported in preserved condition from Brownwood, Texas.

The younger and softer material was processed by an ordinary paraffin method, with tertiary butyl alcohol used as a dehydrating agent. The older branches and roots were sectioned—fresh or after preservation in standard killing fluids—on sliding or freezing microtomes. In general, the methods followed were those previously described in detail (Esau, 1948*a*) and much of the material was stained with Bismarck brown and iodine green and mounted in Karo syrup.

PATHOLOGICAL ANATOMY OF THE GRAPEVINE AFFECTED WITH PIERCE'S DISEASE

Structure of Vegetative Organs of Healthy Vines. The structure of the axis of various ages of healthy grapevines is described in an earlier paper (Esau, 1948*a*). It is briefly outlined here.

At the end of primary growth a seedling stem or a shoot on an old vine have, from inside out, a parenchymatous pith, xylem, cambium, phloem, cortex, and epidermis. The xylem and phloem are arranged in the form of collateral strands separated from each other by wide parenchyma rays. Through secondary growth, new vascular tissues are formed, and the epidermis, cortex, and some phloem are cut off by a cork cambium originating in the phloem (plate 8, *B*). The interfascicular cambium formed in the rays

produces ray parenchyma. From time to time some fascicular cambial initials are converted into ray initials and, thus, rays arise within the vascular strands. All the rays are wide, so that the secondary vascular region, like the primary, is conspicuously broken up into radial blocks by parenchyma cells (plate 6). Since new cork cambia arise year after year in successively deeper layers of the phloem, the latter tissue is periodically sloughed off, and only a relatively small amount of nonfunctioning phloem accumulates in the bark of the axis. When, at the end of the season, cork is formed in a current-year shoot, the latter becomes the "mature cane." Several-year-old branches bearing the cane are called "arms" by viticulturists. The roots are without pith but have rays, many of which are wider than the rays of stems. The cork cambium cuts off some phloem in the root periodically, but less regularly than in the stem.

The secondary phloem is composed of two kinds of cell masses occurring in alternate radial blocks: the rays (transverse system) and the longitudinal system. The latter consists of alternate tangential tissue bands: the sieve-tube bands and the fiber bands (plate 6, *B*, to the left in the photograph; the fiber bands are narrow and dense). The sieve-tube bands contain sieve tubes, companion cells, and phloem parenchyma. Most of the fibers are septate, and all remain alive until they are separated from the stem by cork. Starch storage occurs in phloem parenchyma, ray parenchyma, and fibers.

The secondary xylem also consists of radially alternating blocks of the transverse (rays) and longitudinal systems (plate 6, *A*). The latter is composed of vessels, fibers, and xylem parenchyma. (According to Penzig, 1882, tracheids also occur in *Vitis*.) The largest structures visible in a section of xylem are vessels (plate 6, *A*), but some of these elements are very narrow. Vessels occur singly or in small groups. The superposed vessel members are connected by simple circular or elliptic openings, sometimes by scalariform perforation plates (Penzig, 1882; Ráthay, 1896; Solereder, 1908). The pit pairs between two adjacent vessels are bordered and elongated. Half-bordered pit pairs occur between vessels and xylem parenchyma cells (plate 10, *A*). The narrow thick-walled cells among the vessels are fibers. These are mostly septate, and contain a nucleus in each compartment. Their pits are bordered and have slitlike openings. The xylem-parenchyma cells surround the vessels and are also scattered among the fibers. The rays consist of parenchyma cells only. Many xylem- and ray- parenchyma cells show dark contents because of the presence of tannins (plate 6, *A*). Starch storage occurs in the xylem parenchyma, ray parenchyma, and fibers. Some of the vessels become plugged with tyloses at the end of the season and these structures also store starch. Tyloses are formed by xylem-parenchyma cells and eventually develop thick, pitted walls.

The anatomy of the grape leaf was described by Penzig (1882) and Solereder (1908), and the present observations agree with the reports of these workers. A single-layer epidermis covers the leaf on both sides and the stomata are restricted to the lower epidermis (plate 1, *A*). The leaf is prominently bifacial, having one layer of very long palisade cells (sometimes two layers) and several layers of spongy parenchyma (plates 1, *A*; and 2, *A*).

The palisade cells have rather dense contents (some of which are probably

tannins) which obscure the chloroplasts. The spongy-parenchyma cells also contain darkly staining material, which is even denser than that of the palisade cells. The chloroplasts in the spongy parenchyma are smaller and apparently fewer in number than in the palisade layer.

The spongy-parenchyma cells next to the palisade differ from the other cells of the same layer: the tannin in these cells occurs in the form of small droplets, and the plastids, although relatively small, are abundant and conspicuous (plates 1, *A;* and 2, *A,* at *a*). Some cells within the spongy parenchyma enlarge greatly and become the containers for raphides, the "raphide sacs." The protoplasts of these cells become disorganized, and the crystals remain imbedded in a mucilaginous substance. Other types of crystals also occur in the mesophyll, but their development is not associated with unusual cell enlargement.

The chloroplasts are usually conspicuously flattened, and sometimes cover the palisade wall with an almost uninterrupted layer. They may contain small starch grains (the light dots in some plastids in plate 2, *A*).

The vascular system of a leaf consists of 5 major veins and numerous anastomosing ones of various sizes. Among the major veins the median is the most robust. The large veins have several bundles each (arranged in a circle in the largest veins) imbedded in vein parenchyma. The net of vascular bundles anastomosing in the mesophyll between the large bundles is located in the upper part of the spongy parenchyma (plate 1, *A*). Each bundle is surrounded by a sheath of border-parenchyma cells with very small chloroplasts and usually with almost no tannin (plates 1, *A,* and 3, *A*). These parenchyma cells are elongated parallel to the bundle course (plate 3, *A*). The somewhat larger bundles traversing the mesophyll are not only imbedded in a sheath of parenchyma cells, but are also connected with the epidermis by means of similar cells (plate 1, *A,* at *c*). These connecting cells constitute the vein extensions considered in such detail by Wylie (1943).

Internal Symptoms of Pierce's Disease. The present observations on the symptoms of Pierce's disease generally agree with those of other workers (see the review of literature), except that, contrary to Butler's report, the anatomic effects were found in axes of various ages and not in the one-year-old canes only. In the following paragraphs is a general description of the internal symptoms, whereas the details on the distribution of these symptoms in different parts of the vine and on their development appear in the succeeding parts of this paper.

Anatomic changes induced by Pierce's disease occur in the xylem, the bark, and the mesophyll. Two types of development occur in the xylem: gum deposition in various xylem cells, including the vessels, and occlusion of vessels by tyloses. According to the literature reviewed previously, gum and tyloses may be induced by various diseases and by physiological disturbances, and tyloses are common in normal plants, but the prevalence of these formations in grapes affected with Pierce's disease (see next part of this paper), justifies their classification as symptoms of the disease. These symptoms may be found in the primary and secondary wood in vegetative parts of various kinds and ages. (Floral parts were not included in this study.)

Several photomicrographs illustrate the effect of Pierce's disease upon the

xylem. Plate 4 compares vascular bundles from petioles of a healthy (*A*) and of a diseased (*B*) vine. In the former the younger vessels are all intact, and their lumina are free of any obstructions. The older vessels (protoxylem) have been partly crushed by xylem parenchyma in the process of normal obliteration (plate 4, *A*, at *a*). The bundle from the diseased vine shows gum in several vessels (plate 4, *B*, at *b*). Obliteration of the protoxylem vessels in this bundle has occurred in a normal manner. In addition, some xylem-parenchyma cells in the oldest part of the bundle have undergone several divisions (plate 4, *B*, at *c*).

Such renewal of meristematic activity was encountered only occasionally in the present study, but evidence was obtained that it resulted sometimes in a differentiation of complete vascular bundles with xylem and phloem. In the bundle in plate 11, *E*, such supernumerary bundles appear at *c*. In the median of the three bundles the tracheary elements occur next to the radial row of old vessels, which appears above in the picture, and the sieve tubes are next to the median of the three radial files of vessels.

Gum deposition may occur in all types of cells in the xylem. Plate 7, *B*, shows heavy accumulations of gum in some narrow vessels, many fibers, and some ray cells (below the wide vessels). In this section, gum was evident in the xylem parenchyma, also, but this phenomenon is more clearly illustrated in plate 14, *B*, where the parenchyma cells surrounding the vessel are partly filled with gum. The vessel in plate 14, *B*, contains both gum and tyloses. The latter are responsible for the partitioning of the vessel lumen.

The gum is yellowish brown in fresh sections and, when treated with phloroglucinol and hydrochloric acid, shows different tones of yellow, orange, and red. The latter color is somewhat more brilliant than the red of the lignified walls, probably because of the admixture of yellow in the red coloration of the gum. In sections stained with safranine and fast green, the gum stains in various tones of green, greenish yellow, and red, and mixtures of the three. As with phloroglucinol and hydrochloric acid, the red coloration of the gum obtained with safranine is more nearly scarlet, whereas that of the lignified walls is purplish red. Bismarck brown and iodine green stain the gum a brownish purple (plate 14).

As Bartholomew (1928) has suggested, the variability in staining reactions of the gumlike deposits is probably a reflection of the variability in properties of the substances intermediate between the carbohydrates and the end product of their decomposition—the gum proper. When the latter is present, red coloration is obtained with the phloroglucinol-hydrochloric acid combination and with safranine. At this stage the walls of vessels containing the gum often stain like the gum itself, particularly the pit-closing membranes. The latter, moreover, become more or less swollen. In plate 14, *A*, the two pit-closing membranes in the middle of the figure, extending along the entire sides of the vessels (evidently the pits are much elongated), are thick and are stained a purplish brown, whereas elsewhere the primary walls are thin and light yellow in color. (The vessels in this figure have gum and tyloses. The walls of the latter are closely appressed to the walls of the vessel, except where gum occurs between the tylose and the vessel wall.)

Tyloses occur in vessels of all sizes, and may or may not be associated with

gum in a given section (plates 6, *B*; 7, *A*; and 10, *F*). In longitudinal sections, the tyloses are seen occluding the vessels for long distances (plate 9, *B*). Similarly the gum, where present, fills few or many of the successive members of one vessel.

The development of tyloses was studied in some detail. To all appearances these structures are formed through an enlargement of the pit-closing membranes of the pit pairs located between the xylem-parenchyma cells and the vessels. Unmodified pit membranes are shown in plate 10, *A*, whereas those in plate 10, *B*, and *C*, more or less protrude into the vessel lumina. The membrane appears fairly thick in the first stages of growth (plate 10, *C*), but later becomes thinner (plate 10, *D*).

The nuclei migrate from the parenchyma cells into the tyloses (plate 10, *D*). Often more than one tylose arise from the same parenchyma cell (plate 10, *E*). The behavior of the nucleus in such instances is not clear. As far as observed, the nucleus occurred in only one, the largest of the several tyloses from a given cell. In plate 10, *E*, for example, only the larger of the two tyloses showed a nucleus (its shadow is visible in the photograph). Perhaps the enucleate tyloses do not grow very long—a suggestion supported by the observation that, in thoroughly plugged vessels, small tyloses occur at the bases of the large ones, which reach far into the vessel lumen.

Tyloses obviously arise from many of the cells lining the periphery of a given vessel, and tannin-free and tannin-containing cells take part in this growth (plate 10, *F*). Tyloses arising from the different parts of the vessel circumference eventually touch one another and thus occlude the vessel lumen completely (plates 7, *A*, to the left, and 9, *B*). After they cease to grow, tyloses develop thick walls with simple pits and may become lignified.

The effect of Pierce's disease upon the bark of the cane is associated with the imperfect maturation of the latter. As was mentioned previously, a cane on a healthy vine entering dormancy stores starch in all vascular tissues, and forms a cork cambium inside of the primary phloem fibers (plate 8, *B*). In diseased canes, starch storage is incomplete and cork cambium fails to develop in more or less extended areas (plate 8, *A*).

Since cork formation causes death and browning of tissues outside of the cork, the patches of cane periphery having none of the latter retain live, green cortex and fail to assume the brownish-grey color characteristic of the bark of mature canes (Hewitt *et al.*, 1942). The uneven formation of cork may be observed in old axes also. Plate 9, *A*, shows a section of a cane in the second year of growth with new cork (at *b*) formed only partially and united with the old cork layer at *a*. The phloem itself shows no histologic abnormalities, except that the irregularity in cork formation may cause an accumulation of excessive amounts of nonfunctioning phloem. In severely affected plants there may be a suppression of phloem development, along with that of the xylem.

The leaves from diseased vines show variable degrees of degeneration. The younger leaves appear to be free of abnormalities as long as they show no mottling. The older leaves exhibit the various features described by Butler (1910). Some bundles show occlusion of vessels with gum (plate 1, *B*, at *d*). One or two or several vessels contain gum, or all are occluded. The mesophyll cells develop variable amounts of material that takes up stains readily. This

material, which, according to Butler (1910), contains gum, tannin, and other substances may emerge into the intercellular spaces (plate 1, *B;* note gum in substomatal cavities above *b*). The severely affected (yellow) leaf areas are thin, and appear incompletely differentiated because the intercellular-space system is poorly developed. If the latter is filled with the gumlike material, the thin dense mesophyll differs strikingly from the thick lacunose tissue of the healthy leaves. (Compare *A* and *B* in plate 1.)

The plastids of diseased leaves tend to accumulate abnormal amounts of starch, particularly in the yellow areas. Plate 2, *B*, shows chloroplasts with starch in the palisade and spongy parenchyma. In plate 3, *B*, the starch grains in the border parenchyma of a bundle are particularly large and uneven in shape and size. In leaves stained with iodine the deepest blue appears in the border parenchyma, as though the carbohydrates were dammed off here. The chloroplasts of the yellow areas lack the normal amounts of chlorophyll. In some parts of the mesophyll the plastids degenerate more or less thoroughly. They fuse into amorphous masses, as in beet leaves affected with the mosaic (Esau, 1944), and eventually break down completely. Sometimes entire cells or cell complexes undergo necrosis. The latter symptom is expressed externally as leaf scalding (Hewitt *et al.*, 1942*b*).

The abnormalities just described are irregularly distributed in a given leaf, and the yellow areas show more pronounced degenerative changes than the green. The chloroplasts in the latter develop smaller starch grains, and the gum is less common than in the yellow areas. Thus, there is a quantitative difference between the green and the yellow areas in degenerative changes, but both are abnormal.

Effect of the Disease on Secondary Vascular Tissues in Axes of Various Ages. According to the review of literature, the principal effect of Pierce's disease was to be sought in the xylem, but the irregularities in cork formation suggested that the phloem also might be affected by the presence of the virus in the plant. To obtain some preliminary information on the condition of the phloem in diseased vines, bark samples from eight diseased and five healthy (that is, free from symptoms[4]) vines were collected from a vineyard in Napa Valley on June 14, 1945. The material was sectioned fresh, and was treated with iodine and aniline blue.

The phloem of the diseased and the healthy vines was compared for the number of annual increments present in the bark, the composition of these increments (that is, number of fiber and sieve-tube bands), the thickness of the whole phloem and of its functioning part, and the per cent of functioning phloem (table 1). In addition, the phloem of affected plants was examined for any evidence of specific degenerative changes. No such changes, however, occurred in this set of samples (nor in any other collected for the present study). The features identifying the nonfunctioning part of the phloem were the same in the healthy and in the diseased vines: partial collapse of sieve tubes and presence, in these elements, of residual material, tyloses, and definitive callus; the absence of slime and starch in the cell lumina and of connecting strands in the sieve plates. (See Esau, 1948*a*.)

[4] In field collections there was, of course, no assurance that vines free from symptoms were also free of Pierce's disease virus. Nevertheless, for convenience of expression the check plants are here spoken of as "healthy plants."

On June 14, when the collection was made, radial growth had not yet ceased, and no 1945 cork was present in the samples. The bark contained phloem formed in the past seasons and some 1945 phloem. In most samples, the old phloem was represented by the 1944 increment. Two of the diseased and one of the healthy vine samples contained the 1944 and the 1943 phloem incre-

TABLE 1

CHARACTERISTICS OF THE PHLOEM OF GRAPEVINES WITH AND WITHOUT SYMPTOMS OF PIERCE'S DISEASE

Vine number	Condition of vine	Rating of disease*	Number		Thickness in mm		Per cent of functioning phloem
			Of complete annual increments	Of fiber bands in complete increments†	Of total phloem	Of functioning phloem	
Carignane							
1	Diseased	4	1	2	0.67	0.25	37.3
2	Diseased	4	1	3	0.83	0.70	84.3
3	Diseased	3	1	3	0.82	0.65	79.3
4	Diseased	2	1	4	1.35	0.70	51.9
5	Healthy	0	1	4	1.47	1.27	86.4
6	Healthy	0	1	3	1.32	1.00	75.8
Green Hungarian							
7	Diseased	2	1	4	0.93	0.65	69.9
8	Healthy	0	2	4	1.02	0.75	73.5
Palomino							
9	Diseased	4	1	4	1.00	0.72	72.0
10	Diseased	4	1	4	1.28	0.62	48.4
11	Diseased	3	1–2	4	1.07	0.79	73.8
12	Healthy	0	3	6	1.38	0.70	50.7
13	Healthy	0	1	4	1.18	0.87	73.7
Palomino, 4 separate samples of vine 11							
11	Diseased	3	1	4	1.05	0.75	71.4
11	Diseased	3	1	4	1.15	0.88	76.5
11	Diseased	3	2	4	0.83	0.57	68.7
11	Diseased	3	1	4	1.25	0.95	76.0
Average of diseased		..	1.0	3.5	0.99	0.64	64.6
Average of healthy		..	1.8	4.0	1.25	0.92	73.6

* Rating by Dr. Wm. B. Hewitt. 0, no symptoms; 4, most severe symptoms.
† The number of sieve-tube bands is one, or a fraction of one, higher than the number of fiber bands. (See Esau, 1948*a*.)

ments, and one of the healthy plants had three old increments in the bark. (Item "Complete annual increments" in table 1.) The number of fiber and sieve-tube bands also varied, mainly in relation to the total thickness of the phloem. Similar variations in the number of annual increments and in the number of fiber and sieve-tube bands were observed in the bark of trunks of the healthy *Vitis vinifera* considered in a previous study (Esau, 1948*a*).

The old phloem and the 1945 phloem together gave the values for the total phloem thickness in table 1. More or less of the old phloem was in functioning state, since this tissue was partly reactivated in the early part of the growth season (Esau, 1948*a*). The reactivated phloem and the new (1945) phloem

were measured together for the value of thickness of functioning phloem in table 1. Although the amount of the 1945 phloem differed from sample to sample, the thickness of the reactivated phloem varied even more. This latter variation largely determined the fluctuations in the per cent of functioning phloem.

In the material used for table 1 the phloem from healthy vines was, on the average, somewhat thicker than that from the diseased, but individually some diseased vines showed thicker phloem than the healthy. Moreover, the figures for diseased vine 11 indicate that a given plant may show variations in bark thickness in different parts of the trunk similar to those between samples from healthy and diseased vines. (See Esau, 1948*a*.)

The per cent of functioning phloem in the healthy vines was, on the average, higher than in the diseased. A sample-by-sample comparison, however, shows no constancy in this difference. The sample from the healthy vine 12, for example, had almost as low a per cent of functioning phloem as those from diseased vines with the lowest values for this item.

The variations in the per cent of functioning phloem could result either from irregularities in cork formation, and the consequent excessive accumulation of old nonfunctioning phloem, or from the failure of rather large amounts of the old phloem to become reactivated. In vine 1 (table 1) the low per cent of functioning phloem was caused by a nonoccurrence of reactivation in most of the old phloem: the functioning phloem formed a small part of the rather thin total phloem. The other three vines with uncommonly low per cent of functioning phloem,—two diseased (vines 4 and 10) and one healthy (vine 12)—had very thick total phloem but only a moderate amount of functioning phloem. In these vines the low per cent of the latter tissue resulted from excessive accumulation of old phloem. Probably both phenomena, incomplete reactivation and imperfect cork formation, play a role in producing barks with excessively low per cent of functioning phloem.

The data in table 1 show no correlation between the condition of the phloem and the severity of symptoms on the vine. In the Carignane variety the more severely affected vines had comparatively thin phloem, but the per cent of functioning phloem was not related to the symptom rating. In the Palomino, one of the severely affected vines had a very low, the others a moderate per cent of functioning phloem.

Thus, this preliminary study showed no specific nor constant quantitative differences between healthy and diseased vines with regard to the structure of the phloem, but it indicated that the total and relative amounts of the functioning phloem may be reduced by the disease.

Two subsequent series of studies were designed to determine the condition of both the xylem and the phloem in the diseased vines as compared with the healthy. The first of these two series of studies concerned two- to five-year-old arms of diseased and of symptom-free vines of two field-grown varieties Emperor and Palomino. The Emperor was growing in the San Joaquin Valley, and, since the vineyard was rogued each season, the available diseased vines were mostly those developing the leaf symptoms during the season of sampling, and were only moderately affected. The Palomino vines were growing in the Napa Valley. They were affected by the disease for variable lengths

TABLE 2

XYLEM AND PHLOEM CHARACTERISTICS IN ARMS FROM EMPEROR AND PALOMINO GRAPEVINES WITH AND WITHOUT SYMPTOMS OF PIERCE'S DISEASE

Variety	Symptoms present or absent	Age of arm in years	Number of samples	Thickness in mm of annual rings of xylem in the various years			Thickness in mm of phloem		Per cent of functioning phloem	Per cent of vessels with tyloses in the xylem of the various years		
				1946	1945	1944	Total	Functioning		1946	1945	1944
Emperor	Absent	2 to 4	31	1.90	2.12	2.66	0.70	0.55	78.65	5.77	9.13	14.24
Emperor	Absent	3	28	1.85	2.11	2.78	....	....	.....	.....	.....	.....
Emperor	Present	2 to 5	31	1.75	1.82	2.37	0.65	0.46	70.33	57.80	53.42	41.53
Emperor	Present	3	18	1.84	2.13	2.87	....	....	.....	.....	.....	.....
Palomino	Absent	3 to 5	31	1.30	1.72	2.11	0.51	0.39	77.14	12.41	22.09	29.13
Palomino	Absent	3	27	1.32	1.86	2.27	....	....	.....	.....	.....	.....
Palomino	Present	3 to 5	31	1.50	1.80	2.21	0.56	0.40	70.84	44.17	42.90	36.89
Palomino	Present	3	26	1.59	1.92	2.42	....	....	.....	.....	.....	.....

of time, and consequently showed symptoms of different degrees of severity. The water conditions in the Napa Valley vineyard were less favorable than those in the San Joaquin Valley vineyard.

The collections were made September 18, 1946, from the Emperor variety, and November 2, 1946, from the Palomino variety. In each variety, 31 diseased and 31 healthy vines were sampled. The plants without symptoms were scattered among those showing symptoms, and the two kinds of samples were taken in pairs from vines growing as close as possible to each other. An effort was made to select arms of the same age, preferably those in their third season of growth. The samples, however, proved to vary in age, from two to five years in the Emperor and from three to five years in the Palomino.

The data obtained from these samples were assembled in table 2. The following three kinds of characteristics were determined: thickness of annual rings of xylem formed in each of the three years given in the table; thickness of phloem and per cent of functioning phloem in the total bark thickness; per cent of vessels with tyloses in each of the three latest annual increments.

Each sample listed in the fourth column of table 2 consisted of one half of a circumference of one arm. The thickness of xylem and phloem was measured in five places distributed as evenly as possible through each increment. (The phloem had usually only one complete increment and a small part of another, which was measured together with the complete increment.)

The count of tyloses was made in ten different portions of each xylem increment. Usually all vessels (except the smallest, which were difficult to recognize at the low magnification used) of a block of tissue enclosed between two rays were counted. The total number of vessels in the ten counts ranged from about 250 to over 400. Gum formation was frequently associated with the tyloses. It occurred in cells around the vessels and also in the lumina of the latter. Sometimes the vessels were filled with gum but contained no tyloses. Such vessels were included in the count together with those filled with tyloses.

Table 2 presents the various averages for each lot of 31 samples and also the averages of xylem thickness for the three-year-old arms selected out of each group of 31 samples. Such treatment seemed necessary because usually the first annual increment of a given arm was somewhat wider than the subsequent ones, and the lots that contained a greater number of samples more than three years old were expected to show a relatively lower average value for the width of the 1944 annual ring. The data in the seventh column in table 2 show that this expectation was confirmed. In the lots containing only three-year-old samples the thickness of the 1944 annual increment was greater than that in the mixed lots, and this difference was particularly conspicuous in the diseased material of the Emperor variety, which contained the largest number of samples over three years old.

Judging by the thickness of xylem and phloem, the Emperor variety produced more vigorous growth than the Palomino, but in tylose development the former showed a greater difference between the diseased and the symptom-free vines than the latter. The high per cent of tyloses in the wood of the symptom-free Palomino probably resulted from the somewhat unfavorable soil and water conditions that prevailed in the Napa Valley vineyard.

A summary of the data in table 2 discloses that, in general, the presence

TABLE 3

CHARACTERISTICS OF VARIOUS TISSUES OF PALOMINO GRAPE VARIETY AFFECTED WITH PIERCE'S DISEASE

Vine number and kind of sample	Cork of 1945	Connection between bark and wood	Range of thickness of phloem in mm		Per cent	
			Total	Functioning	Of functioning phloem	Of vessels with tyloses in 1945 xylem
Vine 1, mildly diseased						
One-year-old cane	incomplete	weak	0.23–0.64	0.13–0.44	56.5–68.6	1.0
One-year-old cane	incomplete	weak	0.28–0.64	0.15–0.46	53.5–71.9	4.5
One-year-old cane	incomplete	weak	0.18–0.41	0.10–0.28	55.5–68.3	25.2
One-year-old cane	incomplete	weak	0.23–0.48	0.13–0.33	56.5–68.7	28.1
One-year-old cane	incomplete	weak	0.25–0.59	0.10–0.23	38.9–40.0	29.0
Average	.....	.....	.....	.....	52.2–63.5	17.6
Vine 2, moderately diseased						
One-year-old cane	absent	weak	0.16–0.54	0.08–0.36	50.0–66.6	32.0
Two-year-old cane	absent	weak	0.20–0.51	0.10–0.13	25.5–50.0	50.0
Main arm	incomplete	weak	0.41–1.05	0.28–0.36*	26.7–87.8	59.5
Average	.....	.....	.....	.....	34.1–68.1	47.2
Vine 3, moderately diseased						
One-year-old cane	.....	very weak	.....	.....	.....	13.3
Two-year-old cane	absent	very weak	0.41–1.02	0.15–0.38	36.6–37.2	16.9
Three-year-old arm	absent	weak	0.64–0.79	0.18–0.23	28.1–29.1	38.5
Four-year-old arm	absent	weak	0.77–1.02	0.31–0.38	37.2–40.3	35.4
Trunk	incomplete	very weak	0.67–1.31	0.46–0.54*	35.1–80.6	43.3
Average	.....	.....	.....	.....	34.3–46.8	29.5
Vine 4, moderately diseased						
One-year-old cane	absent	weak	0.26–0.51	0.08–0.20	30.8–39.2	9.5
Two-year-old cane	incomplete	weak	0.41–0.87	0.13–0.31	31.7–35.6	43.8
Trunk	incomplete	weak	0.66–1.62	0.56–0.67	41.4–84.8	40.8
Average	.....	.....	.....	.....	34.6–53.2	31.4
Vine 5, moderately diseased						
One-year-old cane	absent	weak	0.38–0.69	0.18–0.46	47.4–66.7	6.8
One-year-old cane	present	weak	0.15–0.56	0.10–0.51	66.7–91.2	7.5
One-year-old cane	incomplete	weak	0.15–0.48	0.10–0.38	66.7–79.2	9.1
One-year-old cane	present	weak	0.20–0.54	0.15–0.51	75.0–94.4	10.8
One-year-old cane	incomplete	weak	0.61–0.82	0.33–0.46	54.1–56.1	16.8
One-year-old cane	present	weak	0.15–0.51	0.10–0.41	66.7–80.4	17.7
One-year-old cane	present	firm	0.18–0.48	0.13–0.38	72.2–79.2	24.4
Two-year-old cane	present	weak	0.23–0.44	0.18–0.36	78.3–81.8	15.2
Two-year-old cane	present	weak	0.15–0.33	0.10–0.23	66.7–69.7	37.4
Two-year-old cane	present	weak	0.16–0.66	0.13–0.61	81.3–92.4	44.9
Trunk	present	firm	0.67–0.79	0.49–0.69	73.1–87.3	30.9
Average	.....	.....	.....	.....	68.0–79.9	20.1

of symptoms was not associated with any marked depression of growth in the arms as evidenced by the small variations (and these were often in favor of the diseased vines) in the thickness of the xylem and phloem tissues. The per cent of functioning phloem in the healthy vines was slightly higher than

TABLE 3—*Continued*

Vine number and kind of sample	Cork of 1945	Connection between bark and wood	Range of thickness of phloem in mm		Per cent	
			Total	Functioning	Of functioning phloem	Of vessels with tyloses in 1945 xylem
Vine 6, severely diseased						
One-year-old cane	absent	firm	0.15–0.28	0.10–0.18	64.3–66.7	10.5
One-year-old cane	absent	weak	0.20–0.43	0.10–0.28	50.0–65.1	23.4
Two-year-old cane	absent	weak	0.20–0.77	0.10–0.26	33.8–50.0	22.7
Three-year-old cane	absent	weak	0.30–0.80	0.15–0.44	50.0–55.0	19.8
Three-year-old cane	absent	weak	0.51–0.74	0.20–0.28	37.8–39.2	45.1
Trunk	incomplete	very weak	0.66–1.74	0.51–0.72	41.4–77.3	25.8
Average					46.2–58.9	24.6
Vine 7, severely diseased						
One-year-old cane	absent	very weak	0.20–0.51	0.15–0.33	64.7–75.0	32.6
Two-year-old cane	incomplete	weak	0.31–0.92	0.23–0.46	50.0–74.2	67.1
Three-year-old arm	incomplete	weak	0.18–0.92	0.13–0.33	35.7–72.2	59.6
Four-year-old arm	incomplete	weak	0.28–0.52	0.23–0.26	50.0–82.1	59.2
Trunk	uncertain	weak	0.52–0.71	0.26–0.51	50.0–71.9	52.1
Average					50.1–75.1	54.1
Average, vines 1–7					50.8–66.9	29.2
Vine 8, healthy						
One-year-old cane	present	firm	0.28–0.56	0.20–0.46	71.4–82.1	8.9
Two-year-old cane	present	firm	0.25–0.56	0.20–0.46	80.0–82.1	0.2
Trunk	present	firm	0.46–0.64	0.38–0.61	82.6–95.3	0.0
Average					78.0–86.5	3.0
Vine 9, affected with Black Measles						
One-year-old cane	incomplete	firm	0.15–0.48	0.10–0.28	58.3–66.7	3.3
One-year-old cane	present	firm	0.25–0.52	0.20–0.44	80.0–84.5	4.5
One-year-old cane	present	firm	0.08–0.36	0.05–0.28	62.5–77.7	7.3
One-year-old cane	present	firm	0.15–0.39	0.10–0.31	66.7–79.5	7.5
Two-year-old cane	present	firm	0.28–0.62	0.23–0.59	82.2–95.2	0.6
Three-year-old arm	present	firm	0.18–0.56	0.13–0.49	72.2–70.9	0.2
Trunk	uncertain	firm	0.74–0.79	0.56–0.61*	70.9–82.4	0.0
Average					70.4–79.6	3.3

* In these sections the smaller amounts of functioning phloem occurred in the thicker parts of the total phloem. Therefore, in the calculation of the per cent of functioning phloem the higher and the lower values for the latter were considered in reference to the lower and higher values for the total phloem, respectively.

that in the diseased. In contrast, the differences in the per cent of tyloses were striking. These structures were obviously more abundant in the diseased vines than in the checks. Moreover, the symptom-free vines showed an expected increase of per cent of tyloses from the younger to the older wood, whereas in the vines with symptoms this increase occurred toward the younger wood. Thus, table 2 clearly shows that Pierce's disease induces a precocious and excessive tylose development in the affected vines.

In the second series of studies on the effect of Pierce's disease on the xylem and phloem, axes of different ages were compared with one another. The

material for this survey was collected on August 14, 1945, and on September 13, 1945, in vineyards located near St. Helena, California. The varieties Palomino, Carignane, and Petite Sirah were represented in the collections. The diseased vines showed symptoms of different degrees of severity. In addition to those affected with Pierce's disease, two vines showing symptoms of Black Measles (Hewitt *et al.*, 1942*b*) were sampled. Three healthy vines, one from each variety, served as checks.

Most of the data obtained for the Palomino variety are given in detail in table 3. The first column of this table specifies the kind of axis that was represented by the sample, and the other columns give the characteristics that were determined for the axes of various ages. The following items were considered (table 3) : development of new cork; condition of the vascular cambium (column headed by "Connection between bark and wood") ; thickness of phloem, with special reference to the relative thickness of the functioning part of the tissue ; per cent of vessels with tyloses in the xylem formed in 1945.

Table 3 includes data on seven vines that were affected with Pierce's disease, and showed three degrees of severity of symptoms. One vine was mildly affected; four displayed symptoms intermediate between mild and severe ("moderately diseased" in table 3) ; and two vines showed severe effects of the disease expressed in dwarfing of the new season's shoots, pronounced mottling and scalding of leaves, underdevelopment and drying of fruit. Vine 8 in table 3 was free of symptoms of any disease, and vine 9 was affected with Black Measles.

When the samples were collected (August), formation of new cork should have taken place, since, under California conditions, this phenomenon usually occurs in July (Esau, 1948*a*). The item "Cork of 1945" in table 3 shows that the development of new cork was irregular in the diseased vines and that this irregularity occurred in new canes and also in older axes. The 1945 cork was sometimes entirely absent in a given trans-section of the axis, or its development was incomplete, that is, it was present in a part of the section only. Some sections of diseased axes showed normal cork development. The cork was present in all sections from the healthy vine and in most of those from the vine affected with Black Measles.

Divisions had ceased in the vascular cambium in all samples, but whereas in the healthy vines the cambial walls had attained the thickness and firmness characteristic of the dormant state (Esau, 1948*a*), these walls were still thin and weak in parts or in the entire sections from the vines affected with Pierce's disease. Therefore the connection between the bark and wood was firm in healthy axes—the bark had ceased to slip over the wood—whereas it was weak or very weak in diseased vines. The vines affected with Black Measles showed dormant cambium like the healthy vine. The uneven cork formation and the delay in thickening of cambial walls—as well as the scarcity of storage starch mentioned previously—are indications of incomplete development of the phenomena of dormancy. In other words, the axes of diseased vines do not "ripen" properly toward the end of the season.

The one- to three-year-old canes were represented by entire cross sections, but the samples from the arms and trunks consisted of fractions of the circumference. The thickness of the phloem of a given section was measured in

two regions, the narrowest and the widest. A record of the degree of variation between the thinnest and the thickest portions of the phloem was of interest with regard to the data on the activity of the cork cambium: the presence or absence of cork should have something to do with the thickness of the phloem at the end of the season.

According to table 3, the phloem (total and functioning) fluctuated in thickness in healthy and diseased vines. In some sections from the latter, however, the difference between the thinnest and the thickest parts of the phloem was exceptionally great, and the occurrence of such differences in given sections showed no obvious relation to the presence or absence of cork in the same section. Sometimes the difference in the thickness of phloem depended on the irregular cork development; sometimes it was determined by variations in amount of growth from the cambium in the different parts of a given axis.

The per cent of functioning phloem was generally lower in the vines affected with Pierce's disease than in the healthy vine and in the one showing symptoms of Black Measles. The accumulation of the relatively large amounts of nonfunctioning phloem in the vines with Pierce's disease showed, in this series of samples, a relation to cork activity. Where the latter was absent or was formed incompletely (table 3, vines 1 to 4, 6 and 7) the per cent of functioning phloem was lower, on the average, than in those with normally formed cork (part of samples of vine 5; vines 8 and 9). Thus, the presence of relatively large amounts of nonfunctioning phloem in vines affected with Pierce's disease is, at least partly, another expression of the imperfect development of the dormant state. However, nonoccurrence of reactivation in considerable parts of old phloem and a reduction in cambial growth may also reduce the per cent of functioning phloem.

The tyloses were counted in the same manner as in the first series of studies involving secondary xylem. The primary tracheary elements were included in the counts in the one-year-old canes. In table 3 the excessive tylose development is the most conspicuous distinguishing characteristic of the vines affected with Pierce's disease. The per cent of vessels with tyloses varied considerably in the samples from the same vine, whether the axes represented by the samples from a given vine were of the same age or not. Nevertheless, in the majority of samples the per cent of plugged vessels was higher than the highest per cent in the healthy vine and in the one affected with Black Measles. In the vines with Pierce's disease, the older axes often had more tyloses in the 1945 xylem than the younger ones (see also table 5).

The samples of Petite Sirah and Carignane were surveyed like those of Palomino. In addition to the items listed in table 3, the per cent of tyloses was determined for each annual increment, if more than one of these were present, and the thickness of xylem was measured. Rootstocks from two diseased vines of Palomino and from one of Petite Sirah and some diseased sprouts from Palomino rootstock were examined. (The sprouts showed no tyloses and were omitted from tables 4 and 5.) The studies on Petite Sirah and Carignane gave results similar to those on Palomino (table 3), except that in the average of all samples the former two varieties showed a lower per cent of vessels with tyloses than the latter. The data on Petite Sirah and Carignane are not

given in detail. Instead, the figures obtained with the three varieties are summed up in tables 4 and 5.

Table 4 gives the per cent of vessels with tyloses for each vine sampled. The figures obtained from the axes of different ages and from the various xylem increments were averaged for table 4. The values for the rootstocks were calculated separately from those for the aerial parts. The wood increments of different years showed variable per cent of tylosed vessels. Some-

TABLE 4

COMPARISON OF THREE VARIETIES OF *Vitis vinifera* WITH REGARD TO THE EFFECT OF PIERCE'S DISEASE UPON THE XYLEM

Vine number	Kind of material	Condition of the vine	Per cent of vessels with tyloses
Palomino			
1	Aerial axes*	Mild symptoms of Pierce's disease	17.6
2	Aerial axes	Moderate symptoms of Pierce's disease	45.2
3	Aerial axes	Moderate symptoms of Pierce's disease	24.5
4	Aerial axes	Moderate symptoms of Pierce's disease	31.4
5	Aerial axes	Moderate symptoms of Pierce's disease	20.9
6	Aerial axes	Severe symptoms of Pierce's disease	25.3
7	Aerial axes	Severe symptoms of Pierce's disease	46.9
10	Rootstock†	Moderate symptoms of Pierce's disease	27.4
7	Rootstock†	Severe symptoms of Pierce's disease	20.8
8	Aerial axes	No symptoms	5.0
9	Aerial axes	Symptoms of Black Measles	6.4
Carignane			
1	Aerial axes	Severe symptoms of Pierce's disease	22.6
2	Aerial axes	No symptoms	3.6
Petite Sirah			
1	Aerial axes	Mild symptoms of Pierce's disease	27.0
2	Aerial axes	Moderate symptoms of Pierce's disease	20.1
3	Aerial axes	Severe symptoms of Pierce's disease	15.0
4	Rootstock†	Moderate symptoms of Pierce's disease	7.9
5	Aerial axes	No symptoms	5.8
6	Aerial axes	Symptoms of Black Measles	9.9‡

* Only one-year-old canes were collected from vine 1, axes of various ages from the others.
† Palomino and Petite Sirah were grafted on the same kind of rootstock.
‡ The sample from the trunk of Petite Sirah, vine 6, was infected with a fungus and showed tyloses in 62.4 per cent of vessels. This sample was excluded in the calculation of the average for vine 6.

times the value was higher for the older, sometimes for the younger wood, or all increments were similarly affected. (Table 2, however, shows that when sufficient numbers of samples of a similar kind are examined, the normal increase of the per cent of vessels with tyloses from the younger toward the older xylem increments is mostly reversed in the diseased vines.)

According to table 4, excessive production of tyloses under the influence of Pierce's disease is characteristic of all three varieties considered in the table, and it occurs in the rootstocks, as well as in the aerial parts. No rootstock samples were available from the healthy vines in this series, but, according to some other studies on the roots of the grapevine by the writer (Esau, 1948*a*), excessive tylose formation is not characteristic of this organ in healthy plants. The samples analyzed in table 4 are not sufficiently numerous for the reader

to judge the relative resistance of the three varieties, as revealed by the severity of vessel occlusion, or to draw conclusions concerning the comparative effects of the disease upon roots and stems. Table 4 reveals no relation between the severity of the external symptoms and the per cent of vessels with tyloses in the xylem.

Table 5 was compiled from the data obtained with the three varieties considered separately in table 4. The values recorded for the axes of each age were averaged regardless of the variety. According to table 5, in axes of all ages the entire phloem was usually thicker in the diseased vines, but the functioning part of the phloem was wider in the healthy vines; and the per cent of functioning phloem was considerably higher in the check plants. The

TABLE 5

COMPARISON OF THE EFFECT OF PIERCE'S DISEASE UPON AXES OF DIFFERENT AGES OF *Vitis vinifera*

(Combined averages for all the vines given in table 4)

Kind of axis	Condition of vine	Range of thickness in mm			Per cent	
		Of total phloem	Of functioning phloem	Of xylem of 1945	Of functioning phloem	Of vessels with tyloses*
One year old	Diseased	0.23–0.55	0.13–0.37	0.98–1.67	56.5–67.3	15.2
One year old	Healthy	0.19–0.43	0.14–0.35	1.50–2.28	73.7–81.4	3.2
Two to four years old	Diseased	0.32–0.70	0.16–0.36	0.46–1.22	50.0–50.1	34.6
Two to four years old	Healthy	0.29–0.52	0.24–0.44	0.88–2.09	82.8–84.6	0.1
Trunk	Diseased	0.64–1.07	0.44–0.53	1.18–1.51	68.8–49.5	40.1
Trunk	Healthy	0.72–0.84	0.61–0.72	0.68–0.95	84.7–85.7	1.5

* Average for xylem of various ages in axes older than one year.

1945 xylem of the younger axes was thicker in the healthy plants, but the trunk xylem of the same year was wider in the diseased plants. The diseased axes, regardless of age, showed a much higher per cent of vessels with tyloses than the healthy material. Among the diseased samples, the one-year-old canes had relatively fewer tyloses than the older axes.

Thus the studies on axes of various ages taken from commercially grown grapevines all agree that Pierce's disease induces excessive and precocious development of tyloses in the xylem, and more or less reduces the per cent of functioning phloem in the bark. Since the thickness of the bark in the diseased vines is frequently greater than in the check plants, the low per cent of functioning phloem in the former seems to depend mainly on irregularities in cork development—that is, excessive amounts of nonfunctioning phloem are left on the vine because of imperfect cork formation.

Development of Symptoms in Seedlings. This study was planned to serve a three-fold purpose: firstly, to determine the nature of the first anatomic changes in the inoculated plants; secondly, to determine the time interval between the inoculation and the appearance of the first external and internal symptoms; thirdly, to compare the spread of the internal and external symptoms through the plant. Three series of seedlings were used. The first two

(tables 6 and 7) were sampled principally before the appearance of external symptoms; the third (table 8) after such symptoms had developed. All seedlings were grown and treated in a greenhouse.

The plants of the first two series were of different sizes. In series 1 (table 6) the seedlings had three to four leaves above the cotyledons at the time of inoculation (September 8, 1944), and the infective insects were caged on the second leaf above the cotyledons. In series 2 (table 7) the plants had nine to thirteen leaves above the cotyledons at inoculation (September 13, 1944), and the insects were placed on the third or fourth leaves from the top. Ten insects (adult *Draeculacephala minerva* Ball) were placed on each plant for 24 hours. Alfalfa plants affected with Pierce's disease virus served as a source of the inoculum.

TABLE 6

DEVELOPMENT OF SYMPTOMS ON GRAPEVINE SEEDLINGS INOCULATED THROUGH THE SECOND LEAF ABOVE THE COTYLEDONS

Sample number	Number of days between inoculation and sampling	Number of leaves on plant counted at sampling	Internal symptoms in inoculated leaf*	External symptoms	
				On the day of sampling	Thirteen months after inoculation
1	3	5	absent	absent	*present*
2	3	3	absent	absent	*present*
3	..†	4	absent	absent	absent
4	6	4	absent	absent	absent
5	6	4	absent	absent	*present*
6	10	4	absent	absent	absent
7	10	5	asbent	absent	*present*
8	..	4	absent	absent	absent
9	13	5	absent	absent	*present*
10	13	4	absent	absent	*present*
11	17	5	absent	absent	absent
12	17	4	absent	absent	absent
13	..	4	absent	absent	absent
14	24	5	absent	absent	absent
15	24	6	*present*	absent	*present*
16	31	4	*present*	absent	*plant dead*
17	31	6	absent	absent	*present*
18	..	4	absent	absent	absent
19	37	6	absent	absent	*present*
20	37	5	absent	absent	*present*
21	44	6	absent	absent	absent
22	44	5	absent	absent	*plant dead*
23	..	5	absent	absent	absent
24	51	5	*present*	absent	*present*
25	51	5	*present*	absent	*present*
26	58	4	absent	*present*‡	*present*
27	58	5	absent	*present*‡	*present*
28	..	5	absent	absent	absent
29	66	6	*present*	*present*	absent
30	66	5	*present*	*present*	*uncertain*

* If internal symptoms were present, they occurred in the inoculated leaf, but were absent in the other parts sampled.

† The plants for which the item "Number of days between inoculation and sampling" is not recorded are untreated control plants. In each instance, the control plant was sampled together with the two plants listed just above it in the table.

‡ The external symptoms on these two plants were observed 3 days before sampling—that is, 55 days after inoculation.

In series 1 and 2 the first collections were made a few days after the infective leafhoppers were placed on the plants. The samplings were repeated at first every few days, and later once a week (see column "Number of days between inoculation and sampling" in tables 6 and 7). On each day of sampling, two

TABLE 7

DEVELOPMENT OF SYMPTOMS ON LARGE GRAPEVINE SEEDLINGS INOCULATED THROUGH THE THIRD OR FOURTH LEAVES FROM THE TOP

Sample number	Number of days between inoculation and sampling	Number of leaves on plant counted at sampling	Internal symptoms in inoculated leaf*	External symptoms	
				On the day of sampling	Thirteen months after inoculation
1	2	11	absent	absent	absent
2	2	13	absent	absent	absent
3	..†	12	absent	absent	absent
4	5	8	absent	absent	*present*
5	5	13	absent	absent	absent
6	8	14	absent	absent	absent
7	8	14	absent	absent	absent
8	..	11	absent	absent	absent
9	12	13	absent	absent	absent
10	12	13	*present*	absent	absent
11	15	15	absent	absent	*present*
12	15	11	absent	absent	absent
13	..	12	absent	absent	absent
14	19	14	absent	*uncertain*‡	*present*
15	19	11	absent	absent	absent
16	26	15	absent	absent	absent
17	26	16	absent	absent	*present*
18	..	12	absent	absent	absent
19	33	14	*present*	absent	absent
20	33	11	*present*	absent	absent
21	40	12	*present*	absent	absent
22	40	11	absent	absent	absent
23	..	10	absent	absent	absent
24	47	11	*present*	absent	*present*
25	47	12	*present*	absent	*present*
26	54	12	*present*	absent	*present*
27	54	13	*present*	absent	*present*
28	..	11	absent	absent	absent
29	62	12	*present*	*present*	*present*
30	62	11	absent	*present*	*present*
31	74	13	*present*	*present*‡	*present*

* If internal symptoms were present, they occurred in the inoculated leaf, but were absent in the other parts sampled.

† The plants for which the item "Number of days between inoculation and sampling" is not recorded are untreated control plants. In each instance the control plant was sampled together with the two plants listed just above it in the table.

‡ In these two plants the main shoot apices were dead and, therefore, axillary buds were sampled for the study of apical meristems.

diseased plants were collected in each of the two series, and every other sampling day a healthy plant was included in the collection. (The samples from the healthy plants are numbered 3, 8, 13, 18, 23, and 28 in tables 6 and 7.) The samples were taken from four different parts of each plant: 1) petiole of the inoculated leaf; 2) internode below the inoculated leaf; 3) internode above the inoculated leaf; and 4) shoot apex with part of the uppermost elongated internode. The portion of the plant remaining after sampling was left undisturbed, and was examined for the development of external symp-

toms about 13 months after the inoculation (October 8, 1945). The results of this examination appear in the last columns of tables 6 and 7.

In series 1 and 2, internal symptoms occurred in few plants (column "Internal symptoms in inoculated leaf"), and among the parts sampled only the petioles of the inoculated leaves showed these symptoms. The pathologic change was localized in the xylem, and consisted mainly of the presence of gum in vessels of various ages, although tyloses also occurred. When present, the gum commonly extended for considerable distances longitudinally in a given vessel, and therefore many successive serial sections from one piece of petiole showed the symptoms. Occasionally, tyloses and small amounts of gum occurred in a few sections on a slide (in series 2, scattered gum formation was found in two of the healthy plants), but such localized plugging of xylem elements was not interpreted as evidence of the disease. Tyloses that occurred in the oldest xylem of the internodes of some healthy and diseased plants were judged to be normal formations associated with the cessation of function of the first-formed xylem.

No other tissues beside the xylem showed any abnormalities in the infected plants, and the apical meristems of the latter did not differ anatomically from those of the untreated plants, except that in series 2 the apices of two plants had died before the sampling was made (samples 14 and 31 in table 7). In many plants, the older of the two or both internodes showed cork formation that was characterized by no abnormalities. The external symptoms were discolorations and necroses on parts of leaf blades.

According to the data in tables 6 and 7, the internal symptoms developed before the external ones. Some plants having gum in the xylem at sampling time failed to develop any external symptoms 13 months after the inoculation. Sometimes the presence of external symptoms was associated with no anatomic changes in the parts sampled. Among the older seedlings the number of plants that eventually showed internal symptoms was higher than among the younger seedlings, but a higher number of the younger seedlings developed external symptoms. The symptom development was irregular in each lot. No explanation of this behavior may be offered at present. Although the seedlings were not uniform genotypically and were expected to show somewhat variable behavior in their response to infection, they all belonged to the species *vinifera*, which is generally very susceptible to the disease.

In the younger seedlings, the first internal symptom was observed 24 days (sample 15 in table 6), and the first external symptom 55 days after the inoculation (samples 26 and 27 in table 6). In the older seedlings, the first anatomic change was noted 12 days after inoculation (sample 10 in table 7). If the death of the apical meristem in plant 14 in table 7 resulted from the disease, then the first external evidence of the latter appeared in the series of older seedlings 19 days after the inoculation (plant 14, table 7). The external symptoms on the inoculated leaf developed in this series 62 days after the inoculation (plant 29 in table 7).

Judging from the last column in tables 6 and 7, Pierce's disease virus certainly spreads downward from the inoculated leaf: although this leaf and the internode below it were removed at sampling, in many instances the remaining stub of the plant developed external symptoms on the new growth. In two

of the small seedlings (samples 1 and 2 in table 6) the virus was present in the base of the plant 3 days after inoculation. However, in general the virus appeared to move slowly out of the inoculated leaf and was therefore frequently removed with the sample, particularly in the plants sampled in the earlier part of the experiment. (See tables 6 and 7, last column.)

The seedlings of the third series, which were used for a comparison of the development of the external and internal symptoms (table 8), had three to four leaves when they were inoculated. The vectors (ten adult *Neokolla circellata* Baker on each plant) were placed for 24 hours usually on the second leaf above the cotyledons, sometimes on the first. The plants were inoculated on January 17 and 18, 1945. The date of appearance of the first external symptoms was recorded for each plant, and the collections for microscopic study were made at different times after these dates. Since series 1 and 2 included healthy material in developmental stages comparable with those of the plants in series 3, no noninoculated seedlings were included in the latter. When sampled, the plants were dissected as for series 1 and 2; in addition, a piece of the midvein with the adjacent mesophyll of the inoculated leaf was collected from each plant. Furthermore, certain other parts were sampled in some plants.

As in series 1 and 2, the plants in series 3 usually showed the first external symptoms on the inoculated leaf. Sample 16 of series 3 was exceptional in that no external symptoms developed on this leaf. The plants of series 3, which were inoculated in the month of January, developed the first symptoms sooner than the plants in series 1 and 2, which were treated in the early fall. Perhaps the better light conditions in the greenhouse in January (because of absence of whitewash on the windowpanes) as compared with those in the early fall (when the windows were coated with whitewash) speeded up the development of symptoms in series 3.

The seventh column in table 8 indicates the manner of spread of the external symptoms beyond the inoculated leaf. The external symptoms developed not only on the leaves located above the inoculated leaf, but also in the leaf below it. Similarly, the internal symptoms spread downward and upward from the inoculated leaf (eighth column in table 8). These observations, together with the data presented in tables 6 and 7 indicate that the infective principle of Pierce's disease moves downward as well as upward in a plant.

The data on the shoot apices were not included in tables 6 to 8. This was not done because, as was mentioned previously, no abnormalities were detected in the apical meristem or the subjacent region in diseased plants, except, of course, in the instances when the shoot tips became necrotic.

The observation that the earliest symptom of Pierce's disease in grape seedlings occurred in the xylem, that it appeared first of all in the inoculated leaf, and that it developed before the external evidences of the disease, suggests that gummosis of the xylem is a primary symptom. Judging by the location of the first internal symptoms (in the inoculated leaf, and then in the well-developed internodes below it and above) Pierce's disease induces degeneration changes in plant parts that are in advanced stages of development.

Additional evidence that xylem symptoms are primary in nature was

TABLE 8

COMPARISON OF THE DEVELOPMENT OF EXTERNAL AND INTERNAL SYMPTOMS IN GRAPEVINE SEEDLINGS

Sample number	No. of days between the different events			Number of leaves above the inoculated, counted at sampling	Description of external symptoms on the date of sampling		Internal symptoms in certain parts other than the inoculated leaf*
	Inoculation and sampling	Inoculation and appearance of external symptoms	Appearance of external symptoms and sampling		On the inoculated leaf	On leaves other than the inoculated	
1	71	36	35	2	Red discoloration along margin; necrosis at apex	None	None
2	71	42	29	2	Red discoloration along margin and in spots on blade; some necrosis	None	Possibly in internodes 1† and 2
3	70	50	20	2	Necrosis along margin	None	None
4	71	51	20	3	Necrosis along margin	None	None
5	70	52	18	2	Red discoloration and necrosis along margin	None	Possibly in internode 1
6	71	53	18	2	Red discoloration and necrosis along margin	None	Possibly in internode 1
7	70	55	15	2	Red discoloration and necrosis along margin	None	Possibly in internode 1
8	70	59	11	3	Necrosis at apex	None	None
9	71	64	7	2	Necrosis at apex	None	Possibly in internode 1
10	71	64	7	3	Yellowish discoloration throughout	Necrotic spot on leaf −1†; necrosis along margin of leaf 2	In internodes 1 and 2
11	71	66	5	4	Necrosis at apex	None	None

12	70	65	5	2	Necrosis at apex	None	Possibly in internodes 1 and 2
13	83	59	24	5	Necrotic margin	Necrotic margin on leaf −1; necrotic margins and spots on leaves 3 to 6	None
14	84	64	20	5	Necrotic margin	Necrotic margin on leaf 3; necrotic spot on leaf 6	None
15	84	66	18	3	Necrotic margin	Yellowish discoloration of leaf −1; necrotic spot on leaf 3	None
16	83	75	8	6	None	Yellowish discoloration of and necrotic spots on leaves 5 to 7	None
17	83	63	20	3	Necrosis almost throughout	Necrotic margin on leaf 2; one necrotic spot on leaf 3	None
18	83	65	18	5	Yellowish discoloration throughout	Yellowish discoloration of and necrotic margin on leaf 2; necrosis beginning on leaf 3	Possibly in internodes 1 to 4 and petioles 3 and 5
19	92	63	29	6	Necrosis of half of leaf blade	Yellowish discoloration of and necrotic spots on leaves 2 and 3; crinkly, upturned margin on leaf 3; necrotic spots on leaves 5 and 6	In internodes 1 and 2
20	92	63	29	4	Necrosis of large areas	Necrosis at apex of leaf −1; necrotic spot on leaf 3	In internode 1
21	93	53	40	3	Red discoloration along margin	Necrotic spots on leaves 2 to 4	None
22	92	82	10	4	Necrosis at apex	None	None

* The inoculated leaf showed internal symptoms in all 22 plants; they had more or less abundant gum and some tyloses in the sections of midveins and petioles.

† The inoculated leaf and the internode below it were given the number 1. The leaves and internodes above the inoculated leaf were numbered 2, 3, etc., beginning with the lowest. The leaf below the inoculated one was numbered −1.

obtained from studies on xylem degeneration in relation to the feeding punctures of the vectors in some seedlings sampled soon after the viruliferous and virus-free insects had fed upon them. Tyloses and gumlike deposits sometimes occurred in the xylem near the feeding punctures made by noninfective leafhoppers in healthy plants. Moreover, some of the punctured vessels were crushed by wound-reaction growth. If, however, infective leafhoppers fed upon healthy plants, gum and tyloses developed not only near the puncture but also at some distance from it. Plate 5, *A*, for example, shows a 14-day-old feeding puncture (torn in the cambial region by wound-healing growth) made by *Draeculacephala minerva* in a certain bundle, and plate 5, *B*, illustrates the condition of the same bundle 750 microns from the feeding puncture. The gum and tyloses in the xylem in plate 5, *B*, seem to have been formed not merely in response to the injury by the insect, but as a first symptom of Pierce's disease.

Effect of the Disease on New Growth on Old Vines. Six shoots with pronounced leaf symptoms, obtained from four vines of the Emperor variety and two vines of a Muscat variety, were compared with healthy shoots of similar stage of development. The collections were made in the spring and early summer. The apex and pieces from four to seven internodes and leaves were available from each shoot, and in the preparation for fixation the position with respect to the apex was recorded for each severed internode and leaf. In each shoot, the search for internal symptoms was made in the apical portion and in the successively older internodes, leaf blades, and petioles.

Pathologic changes were observed in the xylem and the mesophyll; none was observed in the phloem. The xylem showed mainly an occlusion of some vessels with gum. Tyloses occurred in obliterating elements, probably as part of the normal phenomenon of obliteration, since such tyloses were common in healthy material also. The symptoms in the mesophyll were those described in a preceding part of this paper.

In general, gum development in the xylem was scattered in different parts of the shoot and was not at all severe. It was most common in the protoxylem of the apical part of the shoot, in the young leaves and internodes, and in the minor veins of the older leaves. The internodes, the petioles, and the major veins of the blades from the older parts of the shoot rarely showed gum. The protoxylem of the older shoot parts appeared to have been gummed before the obliteration, but the subsequently formed xylem was normal in appearance. Perhaps the occlusion of the protoxylem is the cause of occasional necrosis of shoot apices in affected plants.

Chloroplast degeneration in the mesophyll was evident only in the older leaves, and here it occurred in spots. Both the green and the yellow-leaf areas showed abnormalities, but the green mesophyll was less severely affected.

PATHOLOGICAL ANATOMY OF ALFALFA AFFECTED WITH THE DWARF DISEASE

Structure of Roots of Healthy Alfalfa. Taproots in the secondary stage of growth, tips of lateral roots, and bases of stems were examined for the effects of Pierce's disease virus upon alfalfa. The tips of lateral roots of healthy plants show the usual primary structure characteristic of dicotyledonous

roots. The cortex is wide, is composed of parenchyma, and covered by an epidermis. The narrow central cylinder has diarch, triarch, or tetrarch xylem, two to four phloem strands, and a parenchymatous pericycle which bounds the periphery of the stele. (Compare Simonds, 1935, and Hayward, 1938, p. 322.) The endodermis is characterized by the presence of Casparian strips. The order of differentiation of the xylem, phloem, and endodermis with respect to the apical meristem is considered in connection with the description of root tips of diseased plants.

The taproot in secondary stage of growth is well described and illustrated by Simonds (1935) and Jones (1928*a*). Like other dicotyledonous roots, this organ of the alfalfa plant loses its cortex in the early stages of secondary growth. The pericycle gives rise to a periderm, with orderly arranged cork cells forming the surface of the root (fig. 1, *A*). Beneath the periderm is the phloem, mostly of secondary origin. Functioning sieve tubes occur only near the cambium; elsewhere these elements are obliterated, and the phloem region contains only storage parenchyma and phloem fibers.

The xylem, as seen in figure 1, *A* (inside of the cambium at *a*) is mostly secondary in origin. Some primary xylem occurs in the center of the root, but in old taproots this region is modified as compared with its original state: the xylem parenchyma is much dilated, and the primary-xylem strands are broken up and partly crushed. The secondary xylem contains vessels of different diameters, parenchyma, and numerous fibers, which are lignified in old roots. The vessel elements are mostly scalariform and reticulate. Both the xylem and the phloem show stranded condition because of the presence of wide parenchymatous vascular rays (fig. 1). According to Jones (1928*a*), the secondary-vascular tissues may be produced in very orderly manner, each season's growth beginning with a fibrous layer and ending with tissue containing the conducting elements. In such roots, the annual increments are readily distinguished when the organs become several years old. The material used in the present study showed no particularly orderly alternation of fibers and other tissue elements.

The alfalfa stem retains its cortex and epidermis in the secondary state. The vascular tissues, at first in discrete strands, later become more or less continuous through the activity of the vascular cambium. A parenchymatous pith is enclosed within the vascular cylinder. Prominent caps of phloem fibers delimit the phloem on the outside. In old stems, active sieve tubes occur only near the cambium. Vessels of different diameters occur in the xylem.

Internal Symptoms of Alfalfa Dwarf Disease. As Weimer (1931, 1936) has stated, the characteristic symptom of alfalfa dwarf is the formation of gum in the xylem (fig. 1, *B*, at *c*; plate 15). The gum occurs in stems (plate 15, *A*), at least in their basal portions, and in roots (fig. 1, *B*; plate 15, *B* and *C*). Longitudinal views show the gum filling the vessels for considerable distances. This substance may occur in the parenchyma and fibers adjacent to the affected vessels. In advanced stages of gummosis, partial dissolution of walls leads to the formation of gum pockets. The phloem shows no abnormalities.

The appearance, staining reactions, and the distribution of the gum in the xylem of alfalfa resemble those in the affected grapevine. Plate 15, *A*, shows several vessels with gum in an alfalfa stem. Some of the gum in this photo-

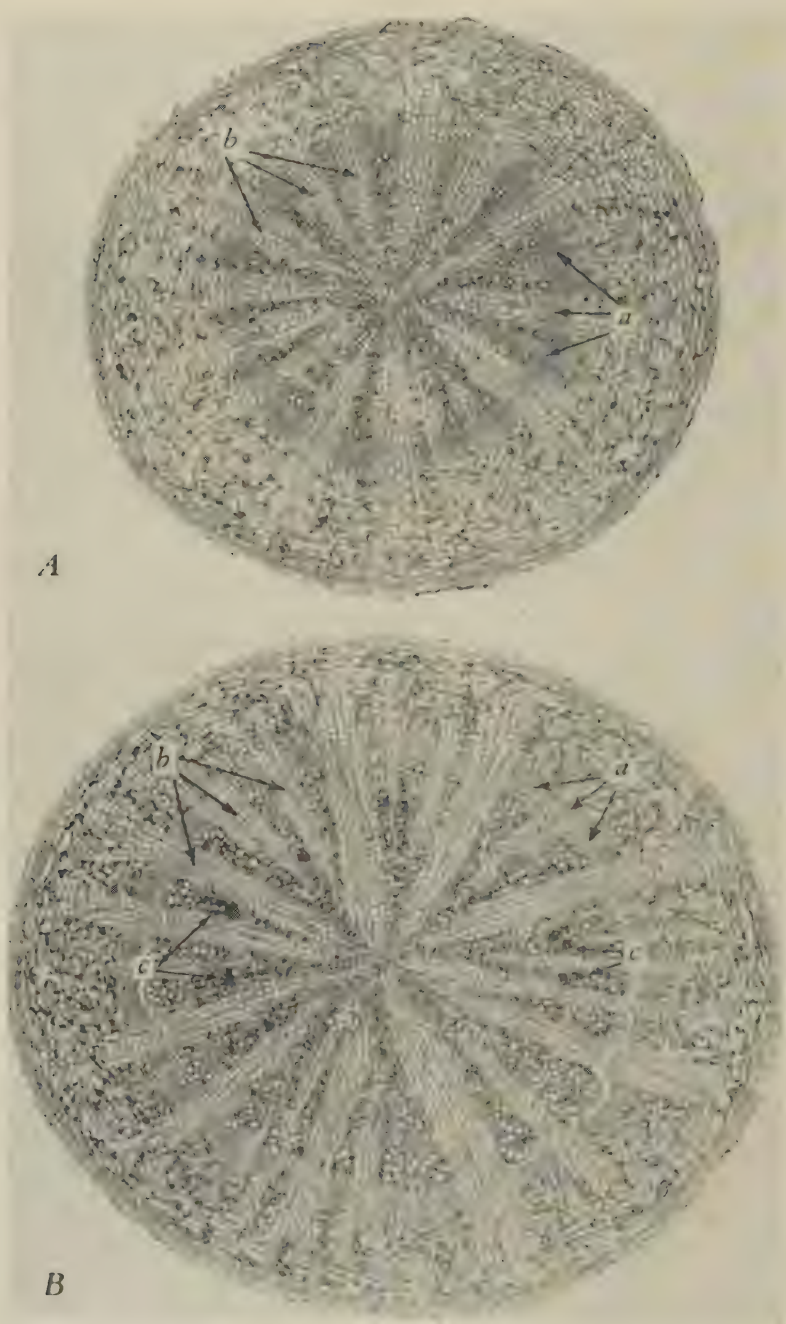

Fig. 1.—Transverse sections of alfalfa roots from a healthy plant (*A*), and from a plant affected with alfalfa dwarf disease (*B*). The regions of the root from outside in are: periderm, secondary phloem, cambium (*a*), secondary xylem, primary xylem. At *b* are some xylem rays. In *B* many vessels are occluded with gum (*c*). (Both × 30.)

graph is stained green and yellow, some red. The latter type of gum is closely appressed to the secondary wall, and shows the same deep staining as the swollen pit-closing membranes which appear like thick red dashes between certain cells. The secondary walls are difficult to discern in the gummed vessels, but wherever visible they show a rather pale coloration. The stains ordinarily used for bringing out lignified walls do not clearly differentiate between the red-staining gum and the similarly tinted walls. Acid fuchsin, however, makes such differentiation possible because it is readily absorbed by the gum and the disorganizing walls, but not so by the apparently unaffected lignified walls. Thus, the views *B* and *C* in plate 15 clearly differentiate between the gum and the swollen pit-closing membranes on the one hand and the secondary wall on the other. Plate 15, *C* also shows, in the lower part of the figure, the disintegration of some parenchyma walls. In the lower right-hand corner of the figure, a swollen wall has pushed part way into the lumen of a parenchyma cell.

To ascertain the causal relation between infection with Pierce's disease virus and gummosis in the xylem, the development of the internal symptom was followed in a series of plants that were sampled at certain known intervals after exposure to feeding by infective vectors. One-month-old plants of the California Common alfalfa variety were transplanted into pots and kept in a greenhouse. When the plants were 2 months old, they were inoculated with Pierce's disease virus by the use of adult *Draeculacephala minerva*. Ten insects were caged upon each plant and were left to feed on it for 2 days. The check plants were left noninoculated, but otherwise were treated just like the inoculated plants. None of the plants developed any external symptoms. Alfalfa affected by the dwarf disease shows no leaf mottling, and the depression of growth characteristic of the disease requires a long time to become evident.

The results of the microscopic studies on the alfalfa plants are summarized in table 9. At successively later periods after the inoculation, series of plants were dug, and root and stem pieces were killed in a mixture of formalin, acetic acid, and alcohol. Each series included two to five plants, mostly four (table 9, first column). The second column in table 9 specifies the contents of the samples from each plant, and the third column gives the observations on occurrence of gum in the xylem of the various samples.

According to table 9, some gummosis was detected 100 hours after the completion of the inoculation procedure. On the succeeding sampling dates, gummosis became more and more pronounced and more widespread. Eight weeks after the inoculation, most of the plants of a series showed severe effects of the disease. One of the control plants (series 10, table 9) had some vessels with gum. The cause of this abnormality was not ascertained, but an accidental infection with Pierce's disease virus was not likely to have occurred because all necessary precautions were taken to prevent the escape of insects.

Thus the data in table 9 clearly show that gummosis in the xylem is associated with alfalfa dwarf, and suggest that it is a primary symptom of the disease.

The root tips were examined for condition of the apical meristem and the course of differentiation of the first vascular elements. There were 118 root

TABLE 9

DEVELOPMENT OF SYMPTOMS IN ALFALFA PLANTS INOCULATED WITH PIERCE'S DISEASE VIRUS

Series and plant number	Parts of plant represented in sample	Vessels with gum
Series 1—20 hours after insects were removed from plant:		
1	Taproot and one stem	None
2	Taproot and four stems	None
3	Taproot and three stems	None
4	Taproot and four stems	None
Series 2—48 hours after insects were removed from plant:		
1	Taproot and four stems	None
2	Taproot and four stems	None
3	Taproot and three stems	None
Series 3—100 hours after insects were removed from plant:		
1	Taproot* and four stems	None
2	Taproot and four stems	None
3	Taproot* and four stems	Few in two stems
4	Lateral root and four stems	None
Series 4—1 week after insects were removed from plant:		
1	Taproot and four stems	Few in two stems
2	Taproot and four stems	Few in one stem
3	Taproot and four stems	Three in root, few in three stems
4	Taproot and four stems	None
Series 5—2 weeks after insects were removed from plant:		
1	Taproot and four stems	None
2	Taproot and four stems	Few in one stem; many in two stems
3	Taproot and four stems	None
4	Taproot and four stems	None
Series 6—4 weeks after insects were removed from plant:		
1	Taproot* and three stems	None
2	Taproot and three stems	Few in root; one in one stem
3	Taproot and two stems	Few in root
4	Lateral root and two stems	None
Series 7—6 weeks after insects were removed from plant:		
1	Taproot and four stems	Many in all parts
2	Taproot and four stems	Few in three stems
3	Taproot and four stems	Few in three stems
4	Taproot and four stems	Few in root and three stems; many in one stem
Series 8—8 weeks after insects were removed from plant:		
1	Taproot and four stems	Many in root; few in four stems
2	Taproot and four stems	Many in root and two stems; few in one stem; very many in one stem
3	Taproot and three stems	Many in root and one stem; few in one stem
4	Taproot and four stems	Many to very many in all parts
5	Taproot and three stems	Few in all parts
Series 9—untreated; plants of same age as in series 6:		
1	Taproot and one stem	None
2	Taproot and three stems	None
Series 10—untreated; plants of same age as in series 8:		
1	Taproot and four stems	One to two in root and three stems
2	Taproot and three stems	None
3	Taproot and two stems	None
4	Taproot and four stems	None

* Each of these taproot sections represented only one half of the entire root. All the other taproots and all stems were represented by entire trans-sections.

tips from diseased plants and 29 from healthy. In both groups, some root tips showed entirely normal structural details, such as dense cytoplasm in the apical meristem; presence of the first sieve tubes at a much shorter distance from the apical meristem than the first xylem; presence of endodermis with Casparian strips at the same level as the xylem. Other healthy and diseased root tips displayed symptoms of inactive growth. The apical-meristem cells were more highly vacuolated than in the first group of root tips; sometimes

TABLE 10

Comparison of Healthy and Diseased Alfalfa Roots with Reference to Starch Storage and Gum Formation

Plant number	Rating of the two phenomena in healthy plants*		Rating of the two phenomena in diseased plants*	
	Starch storage	Gum formation	Starch storage	Gum formation
1	5	0	1	1
2	5	0	0	1
3	5	0	1	2
4	5	0	2	2
5	4	1	0	4
6	4	0	0	4
7	3	0	0	5
8	2	3	2	5
9	4	0	2	3
10	5	0	1	3
11	5	0	3	3
12	5	0	2	2
13	4	1	1	3
14	5	1	0	4
15	3	0	2	4
16	5	0	3	4
17	4	0	1	4
18	5	4	1	4
19	5	0	3	4
20	3	0	2	1
Average	*4.3*	*0.5*	*1.4*	*3.2*

* Qualitative rating: 0, indicating absence; 5, the largest amount of starch or gum.

they appeared hardly less dense than the differentiating cortical cells. The xylem and phloem elements were mature very close to the apex, and both at nearly the same distance from the apical meristem. The xylem elements were of the type usually differentiating in organs that had just ceased to elongate, namely, scalariform and reticulate, whereas in the active root tips the first xylem elements were with spiral thickenings. The endodermis with Casparian strips occurred as close to the apex as the xylem.

The features listed above indicated that some root tips were retarded in their growth, probably because of the topping and transplanting operations. Plants sampled at the latest dates had some root tips in which renewed growth had occurred after a period of retardation. The new growth was clearly set off from the old, and had the normal characteristics of actively growing root tips. In diseased plants, 72.0 per cent of the root tips were more or less retarded, in the healthy, 55.2 per cent. Since the number of healthy root tips

was relatively small, this difference in per cent may not be significant. Four of the 118 root tips from the diseased plants had gum in the xylem. The same abnormality occurred in two of the 29 healthy root tips. There was no evidence of degenerative changes in the phloem in any root tips.

Thus, the root apices of the diseased alfalfa plants used in the present study revealed no anatomic effects that could serve as diagnostic features of alfalfa dwarf. Similar observations were made on the shoot apices of grape seedlings affected with Pierce's disease. Perhaps some special cytological techniques, for example, a staining according to McWhorter (1941), would reveal differences between healthy and diseased plants other than the gummosis in the xylem, but such techniques were not used in the present study.

Since Pierce's disease virus was known to cause a reduction in the amount of starch stored in the canes of the grapevine, a similar effect was expected in the taproots of alfalfa. To check the relation between presence of disease and starch storage in alfalfa, taproots from twenty healthy and twenty diseased field-grown plants were compared for occurrence of starch and gum.

The plants were dug on June 24, 1947, and the roots were preserved in 50 per cent alcohol. The sections were made with a freezing microtome, and stained with iodine for the study of starch deposition and with phloroglucinol and hydrochloric acid for the identification of gummosis. Table 10 summarizes the observations. The amount of starch and gum was estimated qualitatively by using a rating of from 0 to 5 to characterize the roots with the different amounts of these substances. The absence of either gum or starch was indicated by a 0, the highest amounts with a 5. Table 10 shows that most of the roots of the healthy plants contained much starch, but that only a few of these had gum in the xylem. In contrast, the diseased plants stored none or small amounts of starch in the roots, and most of them showed pronounced to severe gummosis. According to the literature, gum is derived to a large extent from hydrolyzed starch. Perhaps some of the starch in the diseased alfalfa roots has been used up in the formation of gum, at least in the immediate vicinity of the gummed vessels. However, it is also possible that the disease adversely affects the elaboration and storage of carbohydrates through disturbances in the water-conducting system. Table 10, at least, reveals no close correlation between the degree of starch depletion and the severity of gummosis.

EFFECT OF PHONY DISEASE ON PEACH ROOTS

As mentioned in the review of literature, the standard test for the identification of phony disease in peach roots—treatment of fresh sections with acidulated methyl alcohol—indicates that some changes occur in the root xylem of affected trees. Following the techniques employed previously in the study of the grapevine anatomy (Esau, 1948*a*), sections of roots from healthy and diseased trees were prepared for microscopic study. Both the xylem and the phloem were examined.

The phloem of the peach has been described in detail by Schneider (1945). The roots from healthy and diseased trees used in the present investigation did not differ in the structure of this tissue. In both kinds of roots the sieve tubes expanded normally in the process of maturation, developed thick (nacré)

walls, and became crushed after their function ceased. The crushed sieve tubes showed no red coloration when treated with phloroglucinol and hydrochloric acid.

According to Burgerstein (1899), the xylem of most Pruneae is diffuse porous, but is ring porous in *Prunus persica*. This feature is probably variable; at least, the peach roots used in the present study had a diffuse porous wood (plate 16, *A*) or a wood that was indistinctly ring porous (plate 16, *B*). The vessels occur singly, or in radial rows, or in groups (plates 12, *B*, and 16, *A*). They have bordered pits and more or less pronounced spiral thickenings over the secondary wall. Strasburger (1891) and Burgerstein (1899) classified the narrow sclerified elements of the xylem (the small thick-walled cells among the vessel in plate 12, *B*) in the representatives of Pruneae as fiber-tracheids and tracheids, respectively, and both authors found no fibers in these plants. The tracheids have bordered pits. The living cells of the peach xylem are largely confined to the rays (plates 12 and 16). The xylem parenchyma is very sparse (plate 13, cells marked with a *b*).

The sections of roots from the phony peach consistently showed variable numbers of gummed areas in the xylem. The gum, granular or homogeneous, partly or entirely filled the vessel and was also present in the tracheids, xylem parenchyma, and ray cells (plates 12, *A*, and 13, *A*). If gum occurred in the ray cells, the latter were partly or entirely depleted of storage starch. (Compare *A* and *B* in plate 12.) In the gummed areas the pit-closing membranes of the water-conducting cells were swollen and of the same color as the gum. (Compare *A* with *B* and *C* in plate 13.) Tyloses occurred in few vessels in the affected areas.

Occasionally gum pockets were present in the xylem, and the orderly localization of these formations in the oldest part of an annual ring (plate 16, *B*, *a*) indicated that degeneration occurred at the beginning of the growth season. (One stem section from a healthy tree showed similar gum pockets in the oldest part of the secondary xylem.) Usually the rays are not destroyed in the formation of the pockets, but often they contain gum in such areas (plate 16, *B*). A similar pattern of gummous degeneration was observed by Webber and Fawcett (1935) in psorosis-affected citrus and by Wigand (1863) in *Prunus avium* suffering from gummosis. Because the gum-containing cavities in citrus are much elongated vertically and anastomose with each other, Webber and Fawcett call them "gum ducts," and suggest that undifferentiated xylem cells are involved in their formation. Butler (1911) characterizes the cells that break down with formation of gum in *Prunus* and *Citrus* affected with gummosis as "a susceptible tissue, in reality embryonic wood cells." According to Wigand (1863), gummous degeneration affects the abnormal strands of parenchyma that are produced by the cambium instead of the normal elements of the longitudinal system. The origin of the gum pockets in phony peach was not investigated in this study. The references just mentioned show that the formation of the orderly arranged gum pockets in the earliest part of an annual ring may be induced by disturbances of various origin, and that it indicates an abnormal behavior of the cambium at the inception of growth in the spring. The size and course of the gum pockets in longitudinal sections were not studied in the peach material.

DISCUSSION

Pierce's disease of the grapevine, alfalfa dwarf, and phony disease of peach may be grouped with the psorosis of citrus as virus diseases causing degenerative changes in the xylem. Moreover, in all four diseases these changes involve formation of gum and its deposition in vessels and in other xylem cells. As was pointed out in the review of literature, gummosis in xylem is not a very specific symptom because it may be caused by various agencies other than viruses.

The details of the gummosis are very similar in the diseases induced by the viruses of Pierce's disease and phony peach. The gum occurs in all types of xylem cells and, if present in the vessels, it fills them longitudinally for considerable distances. (In psorosis-diseased citrus the gum is limited, in the vessels, to the perforation-plate areas.) In the affected xylem of the grape, alfalfa, and peach, the pit-closing membranes often become swollen and discolored. A partial breakdown of cell walls was observed in alfalfa roots, and their complete disappearance, in the formation of gum pockets, in the peach.

The relation of starch to gum has not been clarified in this study. Only the peach material suggested that starch could be the source of gum, since ray cells filled with gum had little starch, whereas unaffected ray cells of the same sections contained abundant starch (plate 12). In all three kinds of plants, the gum showed various gradations of color when treated with phloroglucinol and hydrochloric acid: yellow, orange, and red. With the same reagents, the swollen pit-closing membranes stained a deep red of similar quality as in the red-colored gum.

Whereas in the young axes and in the leaves of the grapevine the deposition of gum in the vessels was the outstanding abnormality, tylose formation was the more striking phenomenon in the older axes. Some few tyloses were observed in the peach, but none in the alfalfa. Since the grapevine readily produces tyloses under the influence of various injuries and in the normal course of ageing of the wood, some specific characteristic of this plant might be responsible for the abundant development of tyloses in response to an infection with Pierce's disease. Ráthay (1896) observed that in cut grape-canes, tyloses developed subsequently to the occlusion of the injured vessels with gum. Perhaps the tylose development in vines affected with Pierce's disease is also a secondary reaction following the formation of gum; at least, the observations on the recently inoculated grape seedlings suggest this sequence of symptom development.

Although Pierce's disease of the grape and dwarf disease of alfalfa are caused by the same virus, the leaf symptoms differ in the two plants: the leaves of the affected grape are mottled, those of the alfalfa are not. No satisfactory explanation of this difference is at present available. The mottling of grape leaves develops in the second or later seasons of infection with Pierce's disease, and may be a purely secondary symptom. Some recent work on virus effects has shown that mottling of leaves may be caused by the virus not directly but through products of a deranged metabolism (Mckinney and Hills, 1941; Wynd, 1943). If the mottling in the grape also is induced by substances other than the virus itself, the absence of such mottling in the alfalfa might be

determined by some distinguishing features of the metabolism in this plant.

In the seedlings of the grape and the cuttings of alfalfa the first symptoms were observed in the more or less developed parts of plants, rather than in the differentiating organs. Although the older literature stresses that viruses induce symptoms mainly in plant parts that are immature when the virus invades them, sufficient evidence has now been accumulated to indicate that such symptoms appear on fully developed organs, also, if the virus succeeds in entering the latter. (See review by Esau, 1948*b*.)

Despite the general similarity of the anatomic symptoms in the diseases caused by the viruses of Pierce's disease, alfalfa dwarf, phony peach, and psorosis of citrus, the tissue relations of these viruses may not be alike. The manner in which Pierce's disease virus is transmitted to its hosts—it must be introduced into the xylem before it can multiply and cause infection (Houston *et al.*, 1947)—clearly points toward a close relation between this virus and the xylem tissue; in contrast, the psorosis virus is readily transmitted through bark grafts (Wallace, 1945). Moreover, the xylem symptoms induced by Pierce's disease virus appear to be primary in origin, whereas characteristic extra xylary symptoms develop more or less in advance of the xylem abnormalities in psorosis-affected citrus (Webber and Fawcett, 1935). Thus, the psorosis virus seems to be less closely associated with the xylem than the virus of Pierce's disease.

As was suggested elsewhere by the present writer (Esau, 1948*b*), the classification of viruses into those occurring in both phloem and parenchyma and those rather restricted to parenchyma or to phloem (Bennett, 1940) may not cover all types of tissue relations of viruses. This is because the association between the causal agent of Pierce's disease and the xylem appears to be as definite as that between the phloem-limited viruses and the phloem tissue.

The exact nature of the relation of Pierce's disease virus to the xylem is not known at present. Presumably it lives and multiplies in the living cells of the xylem. The commonly slow movement of the virus from the inoculated leaf into the other parts of the grape seedling (as evidenced by the late appearance of internal symptoms in the internodes below and above the inoculated leaves and by the large proportion of plants in which the virus was removed with the sample taken soon after inoculation) may have been taking place through the same type of cells. However, the instances of the rapid spread of the virus from the inoculated leaves into the bases of the grapevine seedlings (tables 6 and 7), and from the bases to the tops of the alfalfa plants (Houston *et al.*, 1947) suggest the possibility of occasional movement in tracheary elements. Perhaps the virus is placed by the vector directly into the water-conducting cells—these elements are punctured by the insect during feeding—and if the xylem contents happen to be under tension at this moment, the virus could be drawn rapidly into plant parts rather distant from the place of inoculation.

The relation of the phony disease virus to the xylem is not yet clear. Bennett (1940) places this virus in the parenchyma-limited group, suggesting that it might move and multiply in the parenchymatous elements of the xylem. No definite information is available to explain why the root should be a more favorable habitat for phony disease virus than the stem (Hutchins, 1933, 1939).

The present study revealed no peculiarities in the morphology of the root, as contrasted with that of the stem, which could be used as a satisfactory explanation of the unusual distribution of the virus. It is commonly known that the ratio of living cells to dead cells in the underground organs is higher than in the aerial axes (Liese, 1924; Riedl, 1937; Beakbane, 1941). The peach roots also show more xylem-parenchyma cells and wider and higher rays than stems of similar age and diameter, but the smaller amount of suitable cells does not seem to explain the heretofore reported inability of the virus to make a successful invasion of the aerial parts. If the virus were moving only in the xylem parenchyma and rays, the morphologic and physiologic continuity of the xylem of stem and root and the thorough interconnection among the living cells of the xylem—as revealed by carbohydrate movement, for example—would seem to provide a path along which the virus could enter the stem from the root.

Fulton (1941), however, observed that certain mosaic viruses, which were introduced into roots, caused no infection of the aerial parts of the same plants. The upward movement of these viruses in the roots themselves was very slow, while the downward movement was relatively rapid. Fulton also found that viruses, which were extracted from roots, differed, in certain of their properties, from leaf extracts of the same viruses.

Bennett (1940) has suggested that a combination of a slow movement of the virus and a susceptibility to high temperatures might be the possible cause of the concentration of phony disease virus in the root; no better hypothesis has yet been offered in the literature to explain this phenomenon.

SUMMARY

Naturally infected field-grown material and artificially inoculated greenhouse plants served for the study of the anatomic effects of Pierce's disease of the grapevine and of dwarf disease of alfalfa, both of which are caused by the same virus.

The two diseases induce gum development in the xylem. The gum is deposited in vessels and in other xylem cells. In addition, the grapevine shows a precocious and excessive development of tyloses in the wood.

The xylem symptoms in these diseases appear to be primary in nature. Developmental studies on grape seedlings and on alfalfa cuttings have shown that occlusion of vessels with gum occurs before the development of external symptoms and becomes more and more pronounced with the passing of time after the inoculation. Moreover, in the grape seedling this abnormality appears first of all in the inoculated leaf.

The irregularity in cork formation in the axes and the consequent excessive accumulation of nonfunctioning phloem, which are often found in grapevines affected with Pierce's disease, might be secondary symptoms resulting from the initial disturbance in the water-conducting tissue.

The anatomic changes in affected plants, considered together with the external symptoms and the method of transmission of Pierce's disease virus, suggest a close association of this virus with the xylem tissue.

Peach roots affected with phony disease were obtained from trees growing under field conditions in Texas. All root samples showed gummosis in the

xylem. There was deposition of gum in vessels and other xylem cells and sometimes a formation of gum pockets. Thus, the internal symptoms of phony peach disease resemble those induced by Pierce's disease virus.

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PLATES

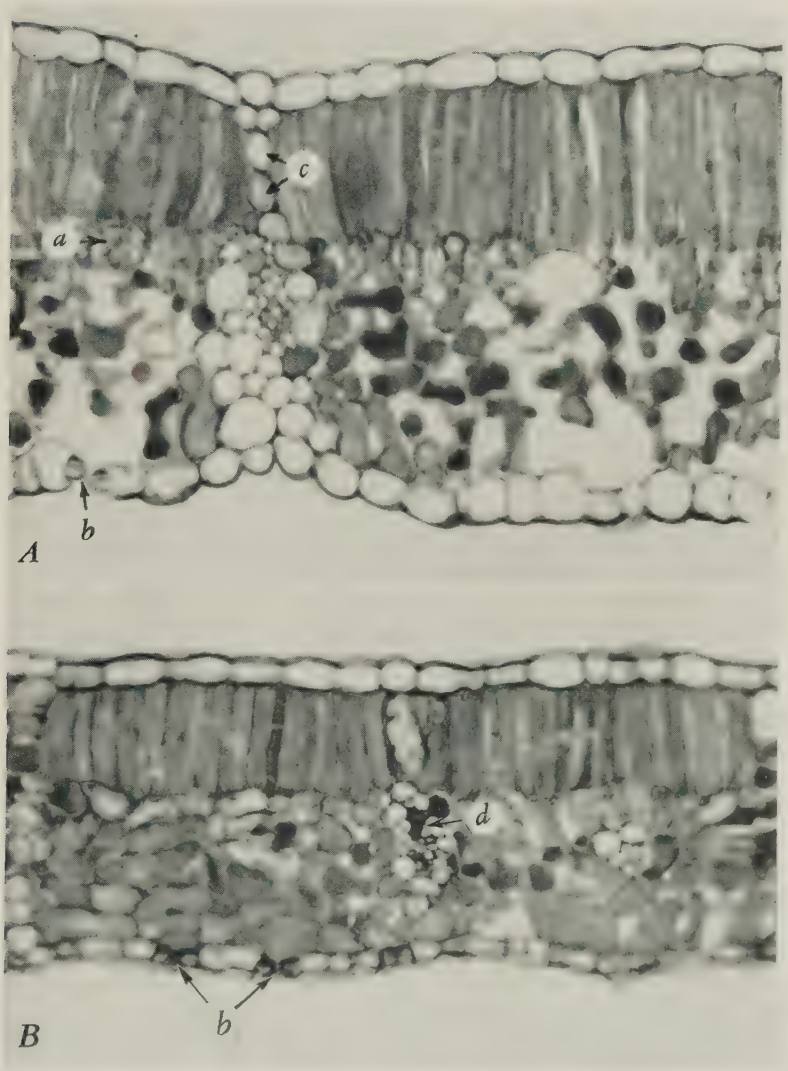

Plate 1.—Transverse sections of grape leaves from a healthy vine (*A*), and from one affected with Pierce's disease (*B*). In both views, the palisade parenchyma appears above, the spongy parenchyma below in the section. In *B*, the intercellular spaces, including the substomatal chambers (above *b*), are filled with a gumlike deposit, and the xylem elements at *d* contain gum. Details are: *a*, uppermost layer of spongy parenchyma; *b*, stomata; *c*, parenchymatous bundle extension; *a*, xylem containing gum. (Both × 290.)

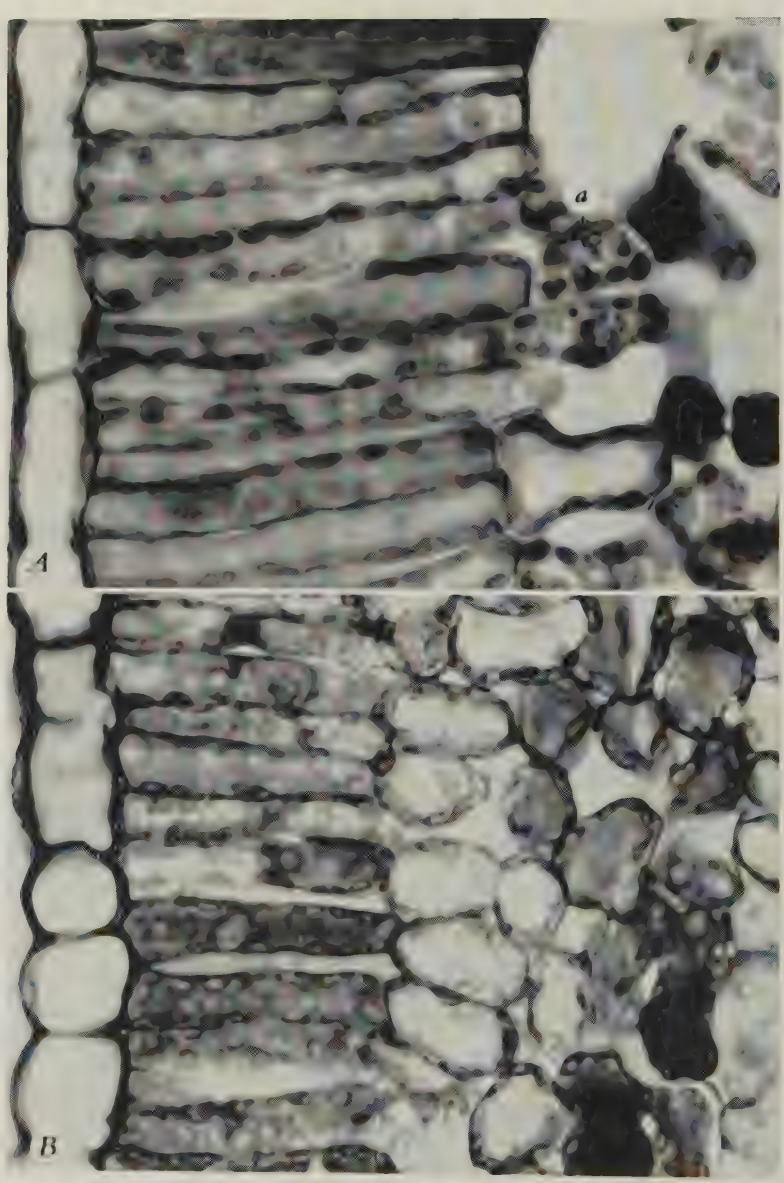

Plate 2.—Transverse sections of grape leaves from a healthy (*A*), and from a diseased (*B*) vine. In both views, the palisade cells appear to the left in the photograph. The mesophyll of the leaf in *B* differs strikingly from that in *A* in having large conspicuous starch grains in the chloroplasts. The diseased leaf also shows somewhat shorter palisade cells than the healthy. Details are: *a*, uppermost layer of spongy parenchyma. (Both × 750.)

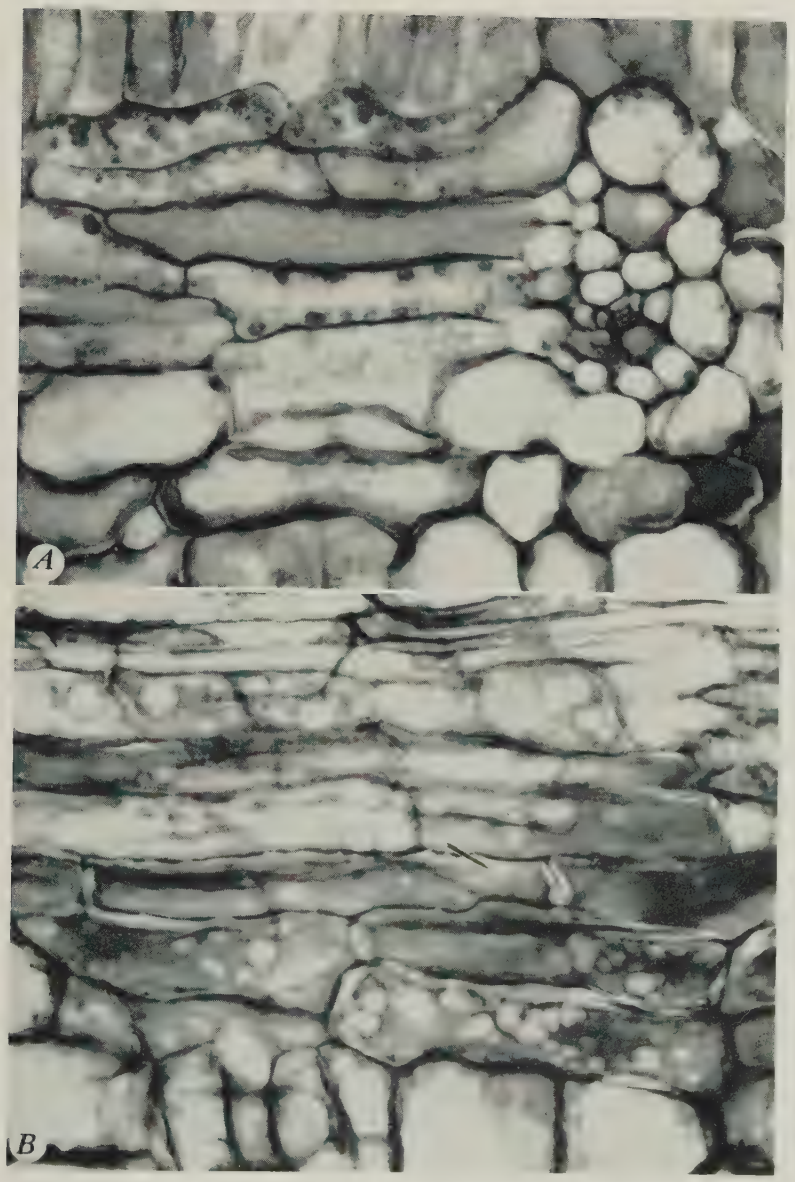

Plate 3.—Transverse sections cut parallel to vascular bundles from a healthy (*A*), and from a diseased (*B*) grape leaf. Both photographs illustrate border parenchyma of the bundles in longitudinal view. This parenchyma shows excessive accumulation of starch in the diseased leaf. (Both × 750.)

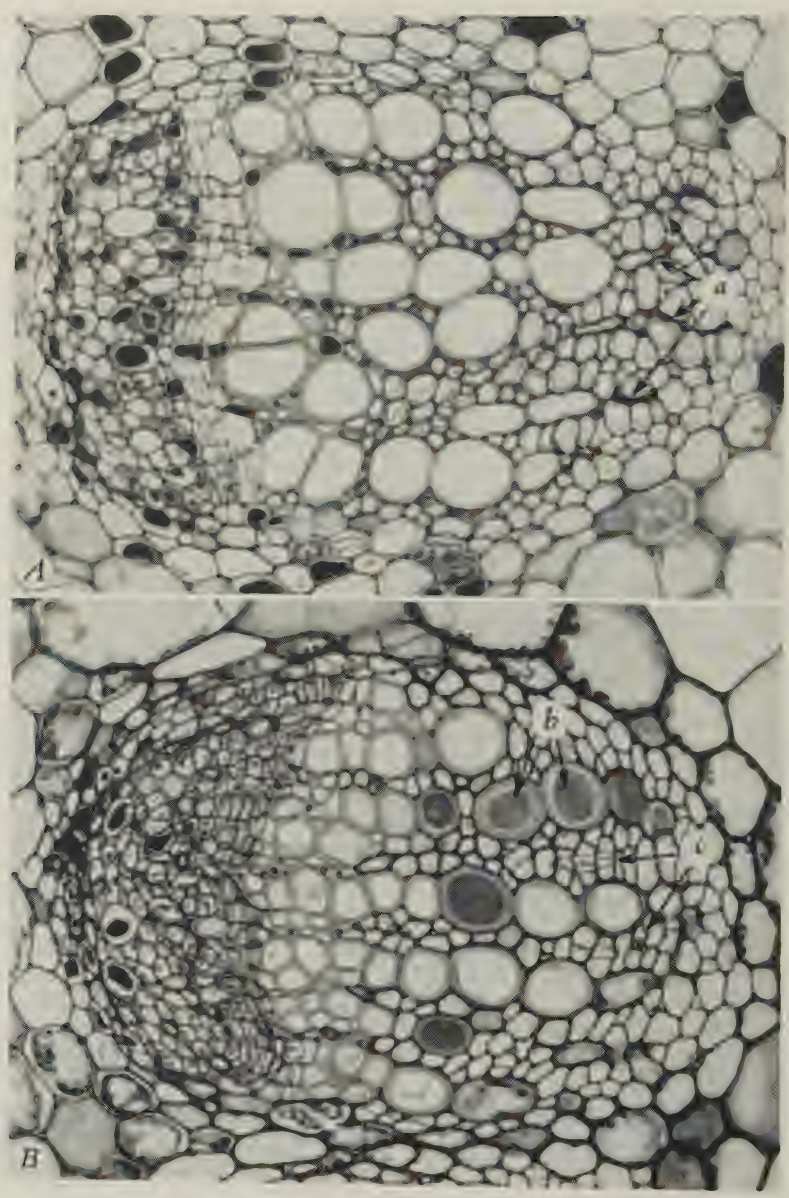

Plate 4.—Transverse sections of vascular bundles of petioles from a healthy grapevine (*A*), and from one affected with Pierce's disease (*B*). In both views the phloem appears to the left, the xylem to the right in the section. Details are: *a*, obliterating protoxylem elements; *b*, vessels filled with gum; *c*, cell division in xylem parenchyma. (Both × 290.)

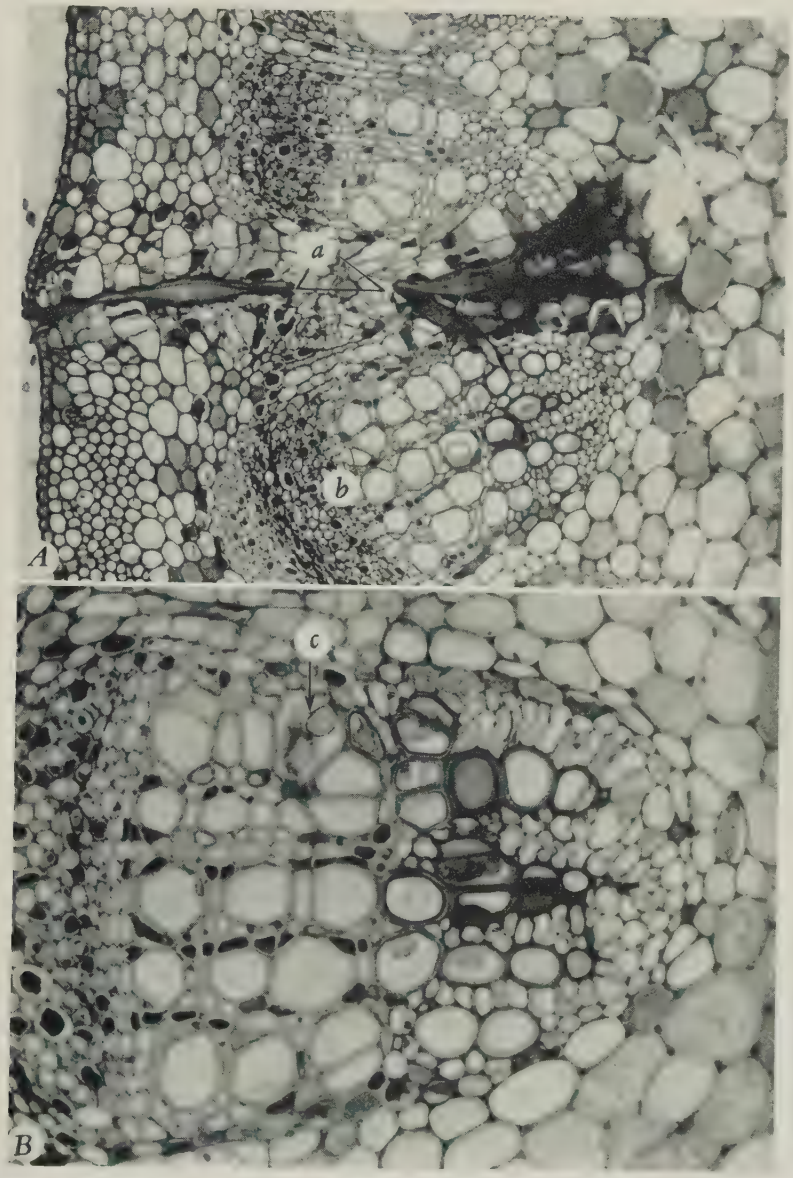

Plate 5.—Transverse sections of a shoot of grapevine sampled 14 days after it was exposed to vectors carrying Pierce's disease virus. An old feeding puncture is visible in *A*. In the cambial region at *a*, the feeding puncture has been healed over. The bundle in *B* is a continuation of the bundle *b* in *A*, taken 750 microns distant from the feeding puncture. It shows vessels with gum in the protoxylem region and a beginning of tylose formation at *c*. (*A*, × 140; *B*, × 290.)

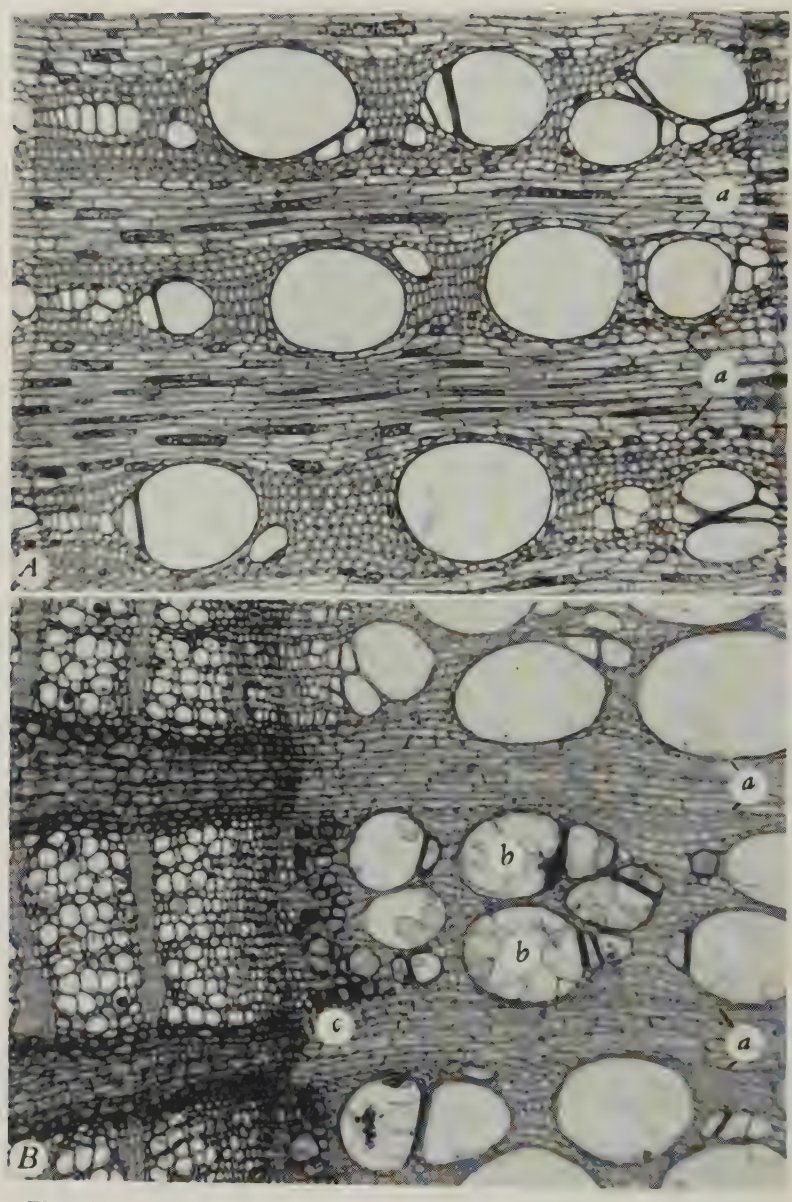

Plate 6.—*A*, Transverse section through the trunk xylem of a healthy grapevine. *B*, Transverse section through the xylem (to the right) and the phloem (to the left) of a cane from a grapevine affected with Pierce's disease. Details are: *a*, vascular rays; *b*, vessels with tyloses; *c*, group of xylem elements (located above the letter *c*) containing gum. (*A*, × 80; *B*, × 90.)

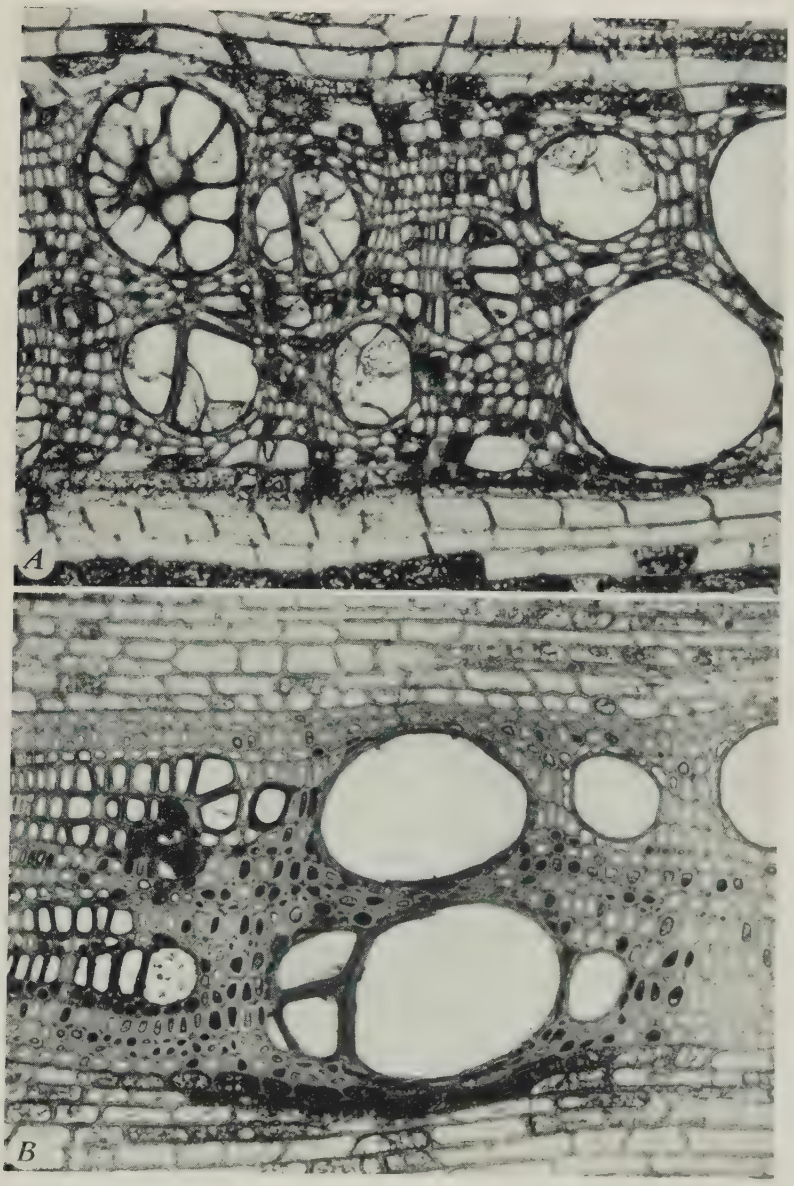

Plate 7.—Transverse sections through a rootstock (*A*), and a cane (*B*) from grapevines affected with Pierce's disease. *A* shows tyloses in the vessels to the left, and *B* illustrates accumulation of gum in various xylem cells including some ray cells (below the large vessels). (Both × 150.)

Plate 8.—Transverse sections through grapevine canes. The cortex is still attached to the cane from a vine affected with Pierce's disease (*A*), but is separated by cork from the healthy cane (*B*). Details are: *a*, primary phloem fibers; *b*, cork. (Both × 90.)

Plate 9.—*A*, Transverse section through a cane in second year of growth from a vine affected with Pierce's disease. At *a* is the cork formed in 1945. The new cork, formed in 1946 (at *b*), does not extend through the whole section, and joins the 1945 cork to the right in the figure. *B*, longitudinal section through the trunk xylem of a diseased grapevine showing vessels filled with tyloses at *c*. (Both × 90.)

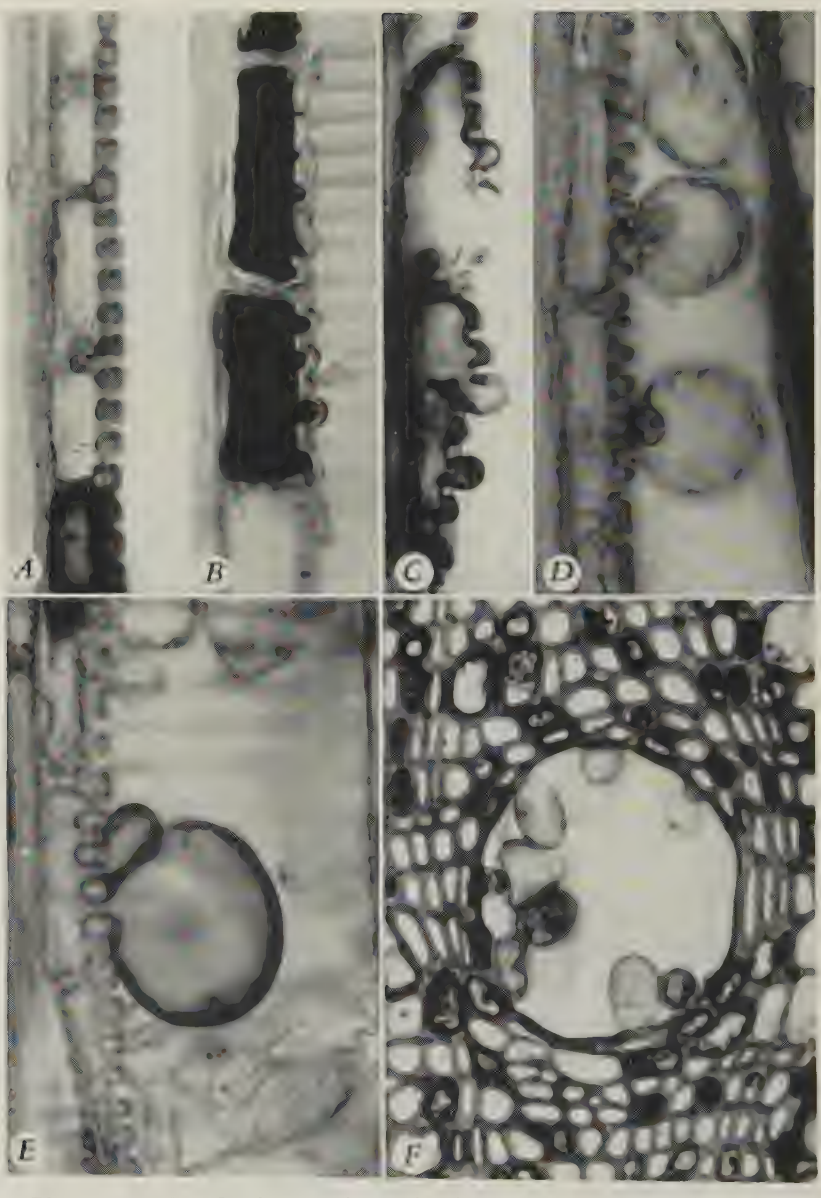

Plate 10.—Tylose development in longitudinal (*A–E*) and transverse (*F*) sections through the xylem of grapevines affected with Pierce's disease. *A*, Xylem-parenchyma cells showing, to the right, the pitting in a wall located between the parenchyma cells and a vessel. *B*, Tannin-containing xylem parenchyma cells in similar position as the cells in *A*. In *B*, the pit membranes are bulging slightly into the vessels. *C*, Similar cells as in *B*, but with somewhat more strongly extended pit-closing membranes. *D*, Young tyloses (rounded cells with nuclei) within the lumen of a vessel, and connected to the parenchyma cells from which they were derived. *E*, Two tyloses formed by the same parenchyma cell from adjacent pit membranes. *F*, Tannin-containing and tannin-free tyloses invading the same vessel. (*A–E*, × 750; *F*, × 150.)

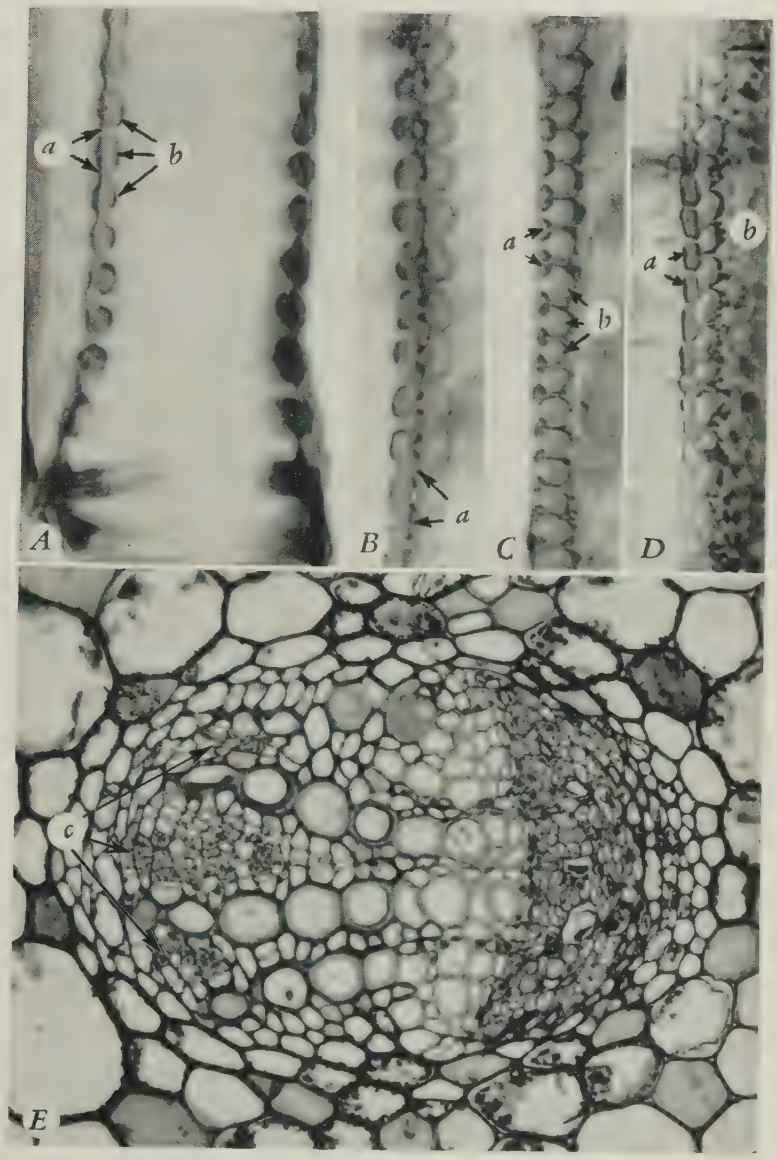

Plate 11.—*A–D*, Vessel walls from a root of alfalfa affected with the dwarf disease. Normal, thin pit-closing membranes appear in unaffected vessels in *A* and *B*. The walls from gummed vessels in *C* and *D* show thickened pit membranes. *E*, Transverse section of a petiolar vascular bundle from a grapevine affected with Pierce's disease. The phloem appears to the right. Details are: *a*, pit-closing membranes; *b*, secondary thickenings of vessel walls; and *c*, vascular bundles that arose within the xylem parenchyma. (*A–D*, × 1200; *E*, × 290.)

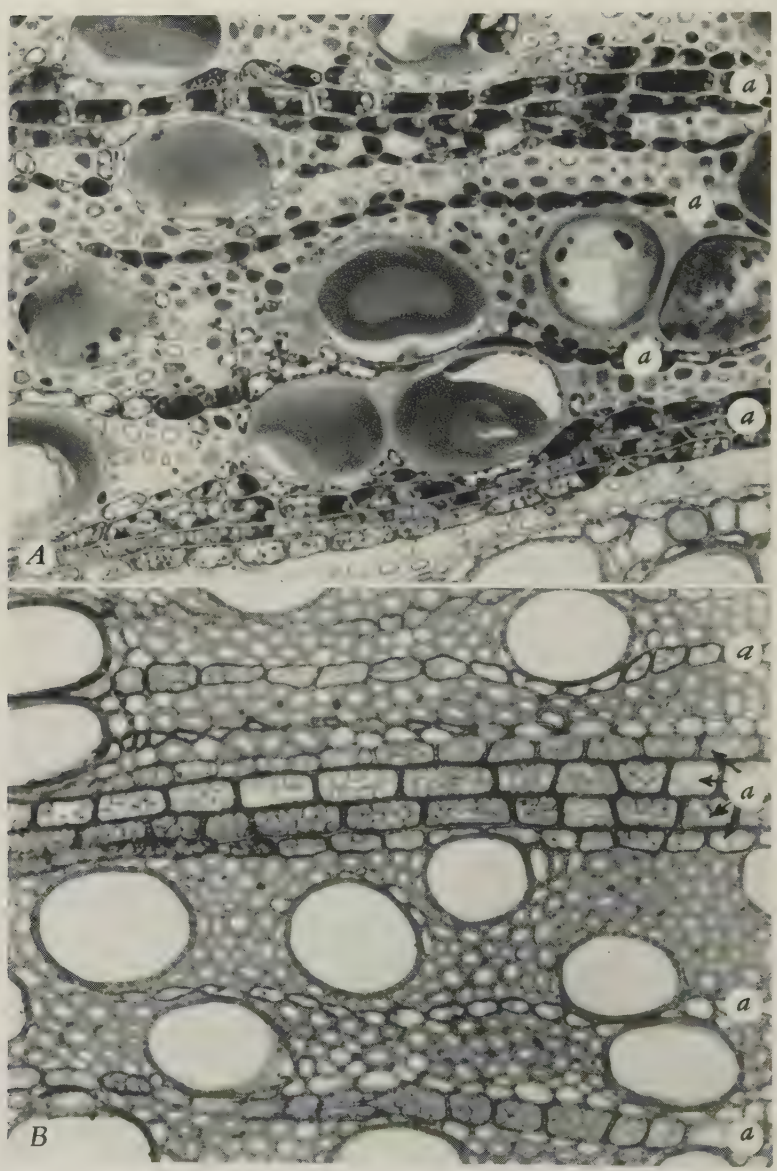

Plate 12.—Transverse sections through a root of a peach affected with phony disease. The area showing gummosis in *A* sharply contrasts with the gum-free area in *B*. Vessels, tracheids, and ray cells (*a*) contain gum in *A*. In contrast to the ray cells (*a*) in *B*, those in *A* show a more or less thorough depletion of starch. (Both × 180.)

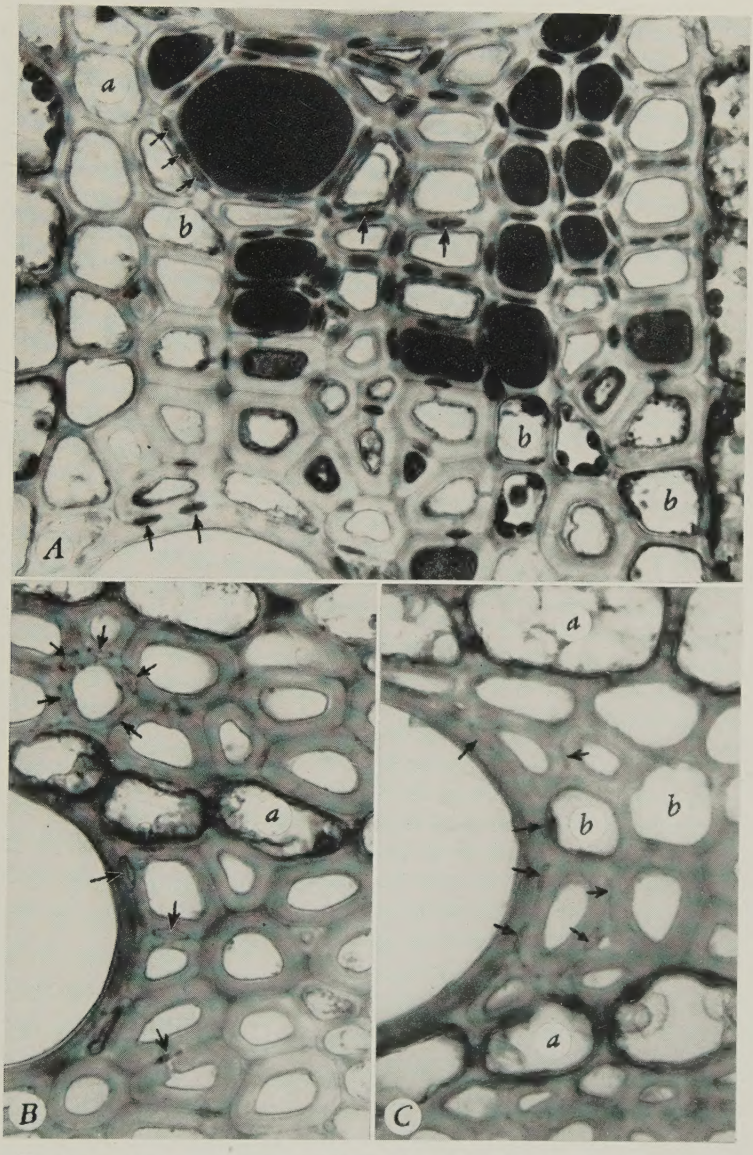

Plate 13.—Transverse sections through a root of a peach affected with phony disease. *A* shows accumulation of gum in many cells and a discoloration and increase in thickness of the pit-closing membranes, some of which are marked by arrows. In *B* and *C* appear sections free of gum and normal, thin pit-closing membranes. Details are: *a*, ray cells; *b*, xylem parenchyma cells. (All × 750.)

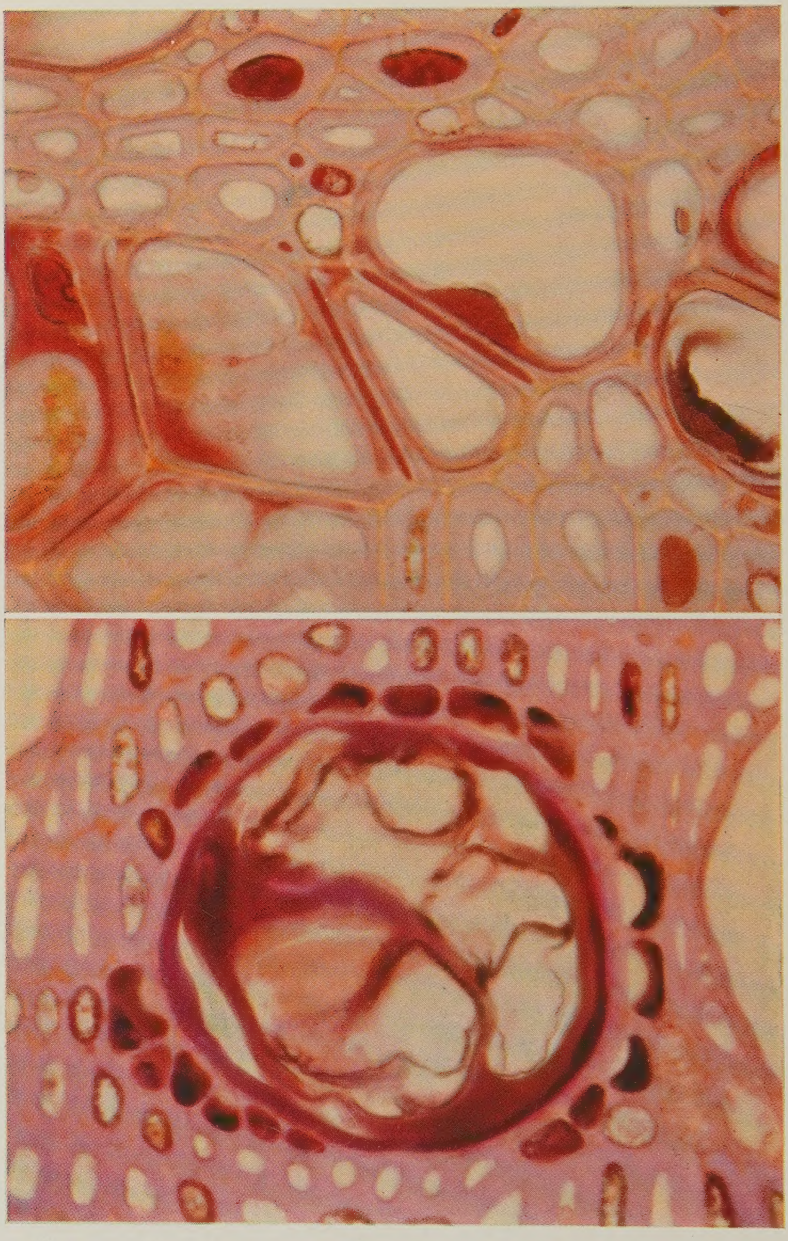

Plate 14.—Transverse sections through the xylem of grapevines affected with Pierce's disease. *A*, Several vessels contain small amounts of gum, and show thickened and deeply colored pit-closing membranes. The walls of tyloses, which are present in these vessels, are closely appressed to the vessel walls and make the latter appear double. *B*, Vessel containing gum and tyloses and surrounded by xylem-parenchyma cells, which are mostly filled with gum. In some of these cells, the gum may be distinguished from the protoplasts. Staining with Bismarck brown and iodine green. (*A*, × 750; *B*, × 720.)

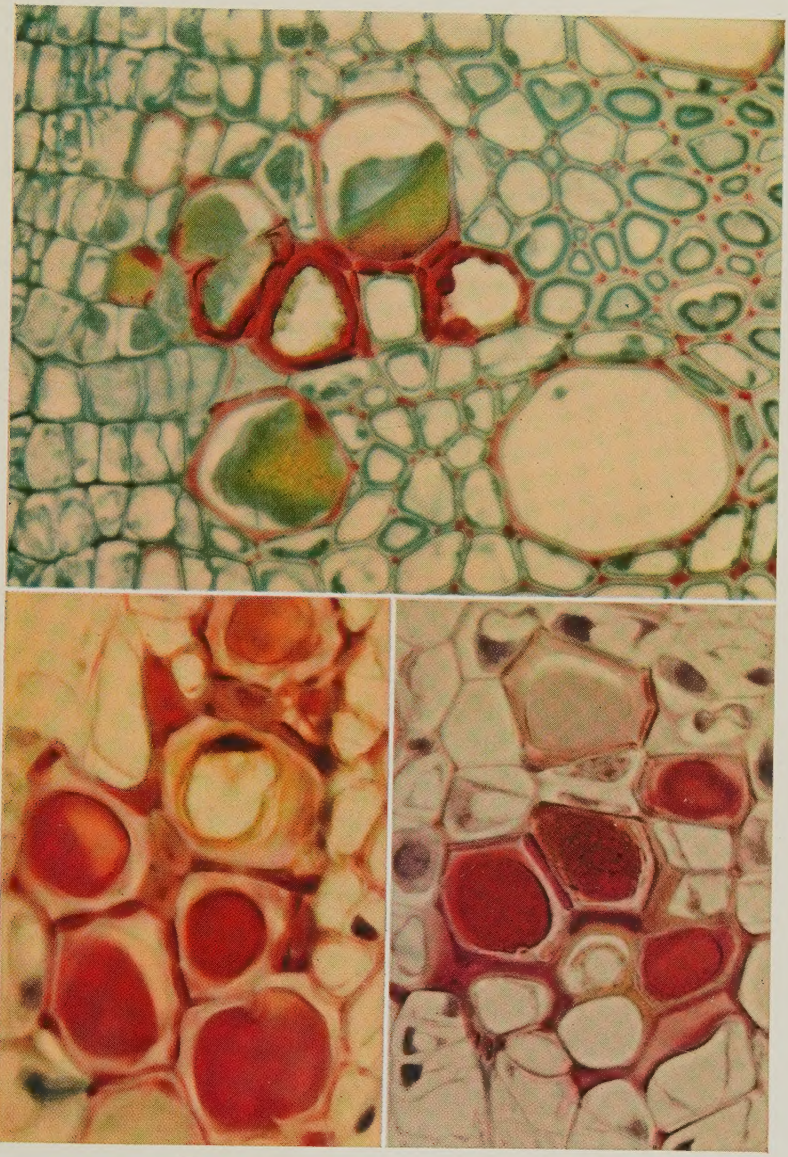

Plate 15.—Transverse sections through xylem of stem (*A*) and root (*B* and *C*) of alfalfa affected with the dwarf disease. *A*, Section stained with safranine and fast green shows at right, below, an unaffected vessel. Other vessels have variable amounts of gum variously stained. The gum, stained red, lines the vessel walls. The latter show thickened pit-closing membranes. *B* and *C*, Sections stained with acid fuchsin. The gum and the swollen pit-closing membranes (very clear in *B*) are deeply stained. Partial dissolution of the primary vessel-parenchyma wall is shown in *C* at right below. (All × 750.)

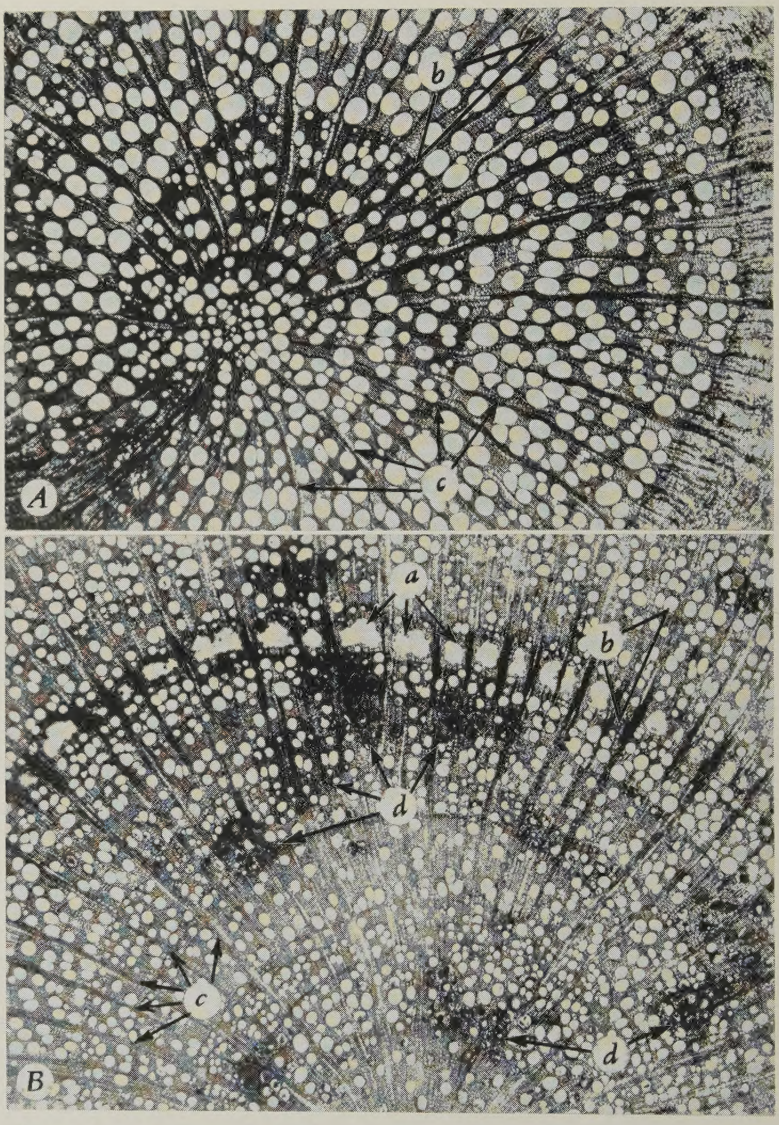

Plate 16.—Transverse sections of roots from a healthy (*A*) and from a phony-diseased (*B*) peach tree. Details are: *a*, gum pockets in the oldest part of an annual ring; *b*, one annual increment of xylem; *c*, xylem rays; *d*, areas containing vessels plugged with gum. (Both × 52.)